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A THEORY OF HISTOGENESIS AND CARCINOGENESIS BASED ON NUCLEAR DIFFERENTIATION

BRUCE E. WALKER*

The multicellular organism develops from the interaction of its cells with each other and with the environment. Assuming a relatively constant environment for a given sequence of histogenetic events from generation to generation, the pattern of cellular interactions is determined by genetic factors residing in the nucleus. The problem to be considered here is how somatic nuclei, which all originate from a single zygote nucleus, bring about the many diverse types of cellular morphology and behavior displayed by cells of the adult organism.

One theory assumes that all nuclei in the organism are genetically identical and that histogenesis takes place because nuclei act differently in different cytoplasmic environments. The evidence on which this theory is based was reviewed by Stern in 1955 (21).

An alternative theory is that nuclei undergo genetic differentiation during the development of the organism. Evidence in favor of this hypothesis has been discussed in a number of recent articles (19, 20, 21, 22, 23); and relates mainly to the following: (a) many nuclei differentiate morphologically (e.g., blood cells); (b) enzymatic differentiation of nuclei has been demonstrated (1); (c) limitations occur in the developmental potentials of nuclei, according to nuclear transplant experiments (3); (d) alterations within chromosomes have been seen in somatic cells (2); (e) somatic polyploidy (an easily visible and relatively permanent change in the genome) has been correlated with cytoplasmic differentiation (23, 24). An article in the Technical Reports section of this issue gives further support to the latter point by showing that polyploidy appears to be essential for normal histogenesis of transitional epithelium after transplantation in the mouse (26).

The problem of whether nuclear differentiation is basic to histogenesis probably will not be settled for a long time. Meanwhile, the concept of nuclear differentiation is just as valid a

working hypothesis as the more prevalent assumption of no nuclear differentiation. The hypothesis of genetically identical nuclei in all somatic cells has been of value in directing attention to the cytoplasm and tissue environment in developmental problems but has not, in itself, contributed to the understanding of genetic control in development. In contrast, the idea of nuclear differentiation seems to provide a basis for understanding some phenomena of cell behavior that are not satisfactorily explained by existing theories on histogenesis.

An explanation of histogenesis in terms of totipotent nuclei has been outlined by Stern (21). He suggests that gene activity varies from negligible to strong depending on what substrates are present, so that although all nuclei in the body have the same genes, some of these genes will be active in one nucleus, whereas a different group will be active in another nucleus if their cytoplasmic environments are different. According to this theory, differentiation is dependent on a stepwise change in the cytoplasm in an environment specifically conducive to this type of differentiation. A loss of cytoplasmic differentiation in an environment quite different from that in which histogenesis originally occurred should then cause the cell to revert to a more primitive type. Thus, the following is a critical question in testing the hypothesis of totipotency in somatic nuclei: If a cell undergoes cytoplasmic dedifferentiation, can it redifferentiate to its former state only, or can it become any one of the many cell types found in that organism?

The degree of cytoplasmic dedifferentiation possible in amphibian limb tissues has been strikingly demonstrated by Hay (7, 8), who wrote: "Heretofore, the possibility could not be excluded that cells retained separate identities and that among blastema cells of diverse origin, morphological differences persisted that were beyond the reach of the light microscope. It can now be stated with considerable assurance that, within the limits of resolution attainable at present with the electron microscope, no such differences exist and that the cells do indeed appear to revert to a single, simplified cell type." (7) After demonstrating that injured muscle fibres contribute cells to the blastema, Hay commented on the matter of redifferentiation as follows: "It seems reasonable to conclude that part of the secret of regeneration of a whole appendage may lie in the remarkable ability of

the formed elements of the limb stump to acquire the embryonic characteristics which allow the cells to proliferate rapidly to form a large mass of undifferentiated cellular material upon which the new limb pattern may be imposed without restriction." (8) This conclusion is reasonable in terms of the totipotent nucleus hypothesis, where a cell's identity is dependent on its cytoplasmic differentiation. However, if a cell's identity is dependent on differentiation of genetic material in the nucleus, then the electron microscope results do not, as yet, bring critical evidence to bear on the question of whether cells can return to a state of broader histogenetic potentialities. The experiments of Hay emphasize the need for tracing a particular cell type throughout the course of dedifferentiation and redifferentiation. This has been done with skeletal muscle regeneration in mice (27). By tracing radioactive nuclei with radioautography, it was shown that nuclei leave the injured muscle fibres, migrate into the wound area, proliferate, line up in rows so that their cytoplasms fuse, and finally differentiate into new skeletal muscle fibres. As far as could be discerned with the light microscope, the cells derived from damaged muscle fibres underwent complete cytoplasmic dedifferentiation, yet they behaved like muscle cells by eventually fusing and differentiating into new muscle fibres. Other cell types were actively involved in the process of wound healing and reconstitution of other tissues, but did not participate in the formation of muscle fibres (27). In fact, it is well known that if damage to skeletal muscle is too extensive, the rapid proliferation of other tissues, especially fibrous connective tissue, will block regeneration of the muscle. Thus, it seems more likely that the extraordinary capacities for regeneration in some amphibia is due to synchronization in the activities of the various cell types rather than a return to totipotency of cytoplasmically dedifferentiated cells. This problem is now being investigated with radioautography in regenerating limb (17, 18) and intestine (18) of the newt.

If the differentiation which determines that a cell becomes a muscle or transitional epithelial cell takes place in the nucleus, then it is obvious that the many special changes in the cytoplasm that lead to the full morphological expression of cell differentiation are not brought about by successive nuclear changes but by the interaction of a special nucleus with various environmental

factors, since the final stages of differentiation are reversible whereas the change from a more primitive cell type by nuclear differentiation is not reversible. Thus, a theory of histogenesis based on nuclear differentiation involves two types of differentiation. Regarding nuclear change, it would occur only occasionally in the life cycle of a somatic cell, as in the case of transitional epithelial surface cells becoming polyploid on the 16th day of prenatal development in the mouse (23). Such a change would establish a completely new behavior pattern for the cell and subsequent differentiation would be the result of interaction between the altered nucleus and the changing cytoplasmic environment. The existence of two degrees of differentiation was recognized by Weiss, who called one "true differentiation" and the other "modulation" (30).

In summary, the type of interaction between nucleus and environment described by Stern seems a necessary component of any differentiation theory. However, the evidence now accumulating that nuclear potentialities decrease during development (3) and that differentiated cells can display a wide range of behavior without losing their identity, despite marked changes in their cytoplasm and general environment (27), indicates that at certain stages of development cells irreversibly change from one behavior pattern to another. The correlation between a genetic change in the nucleus and the initiation of a specific type of cytoplasmic differentiation (23, 24, 26) indicates that these new behavior patterns are the result of nuclear differentiation. Thus, the adult organism would be composed of many cell populations, each with its own genetically specific type of nucleus. Within any one population, the morphology and behavior of the cells could vary due to differential gene action elicited by varying environmental conditions, as proposed by Stern (21, 22), but no environmental influence could change a cell of one population into the cell type of another population (unless, of course, it was a normal stem cell for the other population).

The hypothesis of totipotent nuclei has been quite useless in explaining cancer. However, the situation is very different if the hypothesis of nuclear differentiation is accepted. Cancer becomes an almost necessary sequel to histogenesis, as put forth in the following sequence of reasoning. Consider the appearance of polyploidy in a cell that is normally diploid. If it appears in the

germ line and is transmitted to all cells of a new individual, it will have a marked influence on the development of that individual (4, 6). If it arises in a single somatic cell it will change the behavior pattern of that cell, according to the hypothesis described above. The new behavior pattern of the cell might be advantageous to the species, in which case factors leading to the production of polyploidy in such cells might be established in the species through natural selection. Apparently this has happened in transitional epithelium of the mouse (and presumably in the liver parenchyma of most mammals, too). If the behavior pattern of the polyploid cell was deleterious to the organism, factors preventing the development or survival of these polyploid cells should be established in the species by natural selection. However, once an individual has passed effective reproductive age, or is otherwise a burden to the species (5), changes which take place in tissues of such an aging animal might induce polyploidy in some cells and lead to new, adverse behavior patterns. In this case, protection against such a nuclear change would not have been established by natural selection and the cells would be free to follow their new course of development, which could prove destructive to the organism.

Polyploidy is mentioned here only as one possible form of somatic nuclear differentiation. A variety of other genetic changes that can occur in the somatic nucleus was discussed by Stern (22), Lederberg (14), and others at a recent symposium. Since many types of nuclear changes can take place, many types of behavior patterns could arise, ranging from benign tumors closely resembling the parent cells to malignant forms unresponsive to tissue agents controlling cell behavior (including mitotic rate). The species would not have evolved controlling mechanisms to prevent further nuclear changes in these new cell types, so they could evolve continually further from the cells of origin by successive nuclear changes.

If hereditary protective mechanisms against environmental agents are to evolve, the species must be exposed to these agents over many generations. New environmental agents introduced by man, especially those known to affect the nucleus (radiation, mutagens), could induce nuclear changes leading to a new histogenetic step asynchronous with the welfare of the organism. Viruses constitute another agent that may affect cellular herod-

ity; the relation of the virus hypothesis to the genetic hypothesis of cancer has been discussed by others (10, 20). The idea that cancer is a mutation has been on record for some time. The hypothesis being introduced here is that cancer is due to nuclear differentiation differing from the normal nuclear differentiation of histogenesis only in that it is not a consistent element in the development of individuals toward maximum efficiency for species survival, and so no controlling mechanisms have evolved to synchronize the resulting new behavior pattern with those of other cell types.

Synchronization of behavior patterns must have evolved over a long period of time, considering that cell type took precedent over species type in the experiments of Moscona (16) and Wilde (31). They mixed cells from mouse and chick in tissue culture and discovered that the cells became segregated according to tissue type but remained quite mixed in regard to species type.

Chromosomal studies on cancer cells and cells in tissue culture have produced results that appear to support the theories of nuclear differentiation and carcinogenesis outlined above. Frequent, extreme aneuploid variations in chromosome numbers do not occur in normal somatic tissues of mammals (28). The possibility of subtle degrees of aneuploidy in normal tissues is still being debated (9). In contrast, aneuploid chromosome numbers are very common for cells in tissue culture (12, 15). The obvious interpretation of this difference is that the normal, intact organism provides an adverse environment for the production or survival of aneuploid cells. It is unlikely that a mechanism for exactly reproducing a radical form of aneuploidy in each generation could evolve in a species and therefore it is unlikely that this type of nuclear change would be an agent in normal histogenesis. However, cell populations on which natural selection had not previously been operating, such as populations of tumor cells, would not be subjected to special checks on aneuploidy. Hsu (9) states "When we take a glance at the literature on the karyology of neoplasms, we can immediately notice that it would be difficult to search for a neoplasm that did not contain chromosomal anomalies." Thus, the way would be open for different nuclear changes to appear separately, or in sequence, until the behavior pattern of a malignant cancer cell was produced. The different survival rates of the various aneuploid cell populations

competing in tissue culture (15), and changes in these relative rates with changes in the medium (11), provide evidence for considering that aneuploidy (which is one form of genetic nuclear differentiation) does change the behavior pattern of a cell. The possible causal relation of chromosomal changes in tumor cells and tissue culture cells to malignancy has been discussed at length by others (9, 12).

During the histogenesis of transitional epithelium in mouse embryos, polyploidy arises at mitosis by the fusion of metaphases in binucleate cells (23). Perhaps most cases of nuclear differentiation take place at mitosis or during DNA synthesis prior to mitosis. The divergent fates of stem cells and differentiating cells can be associated with division; for example, when basal cells of stratified squamous epithelium in the vagina divide, one cell remains behind as a basal cell whereas the other moves away and develops into a spinous layer cell (25). Although such histogenetic sequences are very common (29), it is not yet known whether nuclear differentiation occurs at that particular time. However, if the somatic nucleus is especially susceptible to genetic alterations during DNA synthesis and chromosome movements, then the observation of Leblond et al. (13) that sites of primary carcinomas correlate to a large extent with sites of cellular renewal (where there is continuous mitotic activity) could be explained by the hypothesis that cancer arises from aberrant nuclear differentiation. A similar interpretation could be made of the observation that chemical irritants, chronic infections and excessive hormonal stimulation tend to increase mitotic activity in the tissues involved and at the same time increase the probability of tumor production.

Summary

In view of recent evidence supporting the idea of nuclear differentiation being involved in histogenesis, an hypothesis has been presented which is essentially as follows: At intervals during the course of histogenesis, most cells will develop alterations in the genetic material of their nuclei. Such alterations will be relatively irreversible and will establish new behavior patterns for these cells. Within any given behavior pattern, changes in cell activity or state of cytoplasmic differentiation will take place

through the interaction of a genetically stable nucleus with a fluctuating cytoplasmic environment, and these changes will be reversible.

On the basis of this theory, and with reference to recent work on chromosomes of tissue culture and cancer cells, the following hypothesis for carcinogenesis has been offered: A cancer cell develops from the same kind of irreversible nuclear change and consequent modification of cell behavior that occurs in normal histogenesis. It differs from the normal only in that the circumstances under which such a change occurred within the organism were not equivalent to those circumstances in the progressive development of the individual that had been associated with the evolutionary improvement of the species. Therefore, the behavior patterns of cancer cells and their ability to undergo further nuclear alterations have not been limited to a course beneficial to the organism by natural selection.

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ETIOLOGICAL RESEARCH IN THE LIGHT OF THE FACTS OF INDIVIDUALITY

ROGER J. WILLIAMS

This presentation is limited to the research aspects of etiological research and the problems arising out of the existence of a high degree of individuality in all members of the human population. Biochemical individuality and its implications for medical research has already been discussed (Williams, 1956). The present article is an extension of the ideas already set forth and their application, for the sake of concreteness, to a particular group of diseases—muscular dystrophies. The clinical aspects including the practicalities of treatment and care are not discussed because they are quite outside the writer's field of competence or experience.

The point of view which I shall develop departs from concepts which are sometimes regarded by medical writers as axiomatic. The essential departure consists of an altered and more critically defined concept of normality coupled with emphasis on the signal importance in etiological research of the disconformity patterns exhibited by individuals. These have important implications in connection with discovering the nature of disease and in its diagnosis and treatment. In conclusion there is a discussion of cellular nutrition and the outstanding possibilities of prevention and treatment that lie in this area.

Norms in Etiological Research

The unorthodox views with respect to normality can best be introduced by considering the interpretation of currently available data on plasma cholesterol values. Various ranges of what are regarded normal values may be found in different sources as shown in the accompanying table.

The norms derived in the traditional manner from data of this sort can be worthless and misleading in my opinion, when used in etiological research. If one were to include in the "normal range" the values derived from everyone, whether sick or well,

TABLE 1

"Normal" plasma cholesterol values

Author	Date	Number of individuals	Range	Remarks
Page *et al.*	1935	67	112–428	Complete Range
Sperry	1936	59	132–392	Complete Range
Barker	1939	200	135–370	Complete Range
Peters & Mann	1943	174	107–320	Complete Range
Keys *et al.*	1950	145, Age 20	99–248	"Fiducial" limits 98 per cent of population
		90, Age 55	148–364	
Albritton	1952	. . .	105–199	95 per cent of population

this would obviously be based upon the untenable assumption that plasma cholesterol values are completely without etiological significance. If, considering only "well" individuals, we were to accept the 98 per cent level as normal, we would thereby assume without any defensible reason that only 2 per cent of the supposedly well population have cholesterol values that are meaningful with respect to their present or future health. Thompson and King (1957) go so far as to state that "there is no evidence suggesting that such a figure [serum cholesterol] is of any use as a guide either to diagnosis or prognosis of individual cases."

The study of cholesterol values, if this statement is true, results in futility so far as diagnosis or prognosis is concerned. In my opinion the statement is a reflection upon the manner in which cholesterol values have been studied rather than upon their inherent significance. The basis for this opinion will be made clear in later discussions.

From the research point of view the carrying over of the idea of normality and the application of the term *to individuals* is completely indefensible. Every individual human being whether well or diseased possesses a distinctive pattern of anatomy, physiology, and biochemistry. These patterns often involve not trifling details but inter-individual variations of great magnitude. In the case of measurable items, specific inter-individual variations among well individuals may be over a several fold range, as exemplified by the plasma cholesterol values already cited.

The separate items which make up an individual's pattern can each be handled statistically. Averages—means, medians and modes—standard deviations and standard errors, etc., apply and

can be calculated for each if sufficient data are available. Norms for individual items, whether highly useful or not, can be set up at any desired level—95 per cent, 98 per cent, or 99.8 per cent. It is *quite impossible*, however, in a comparable manner, to set up norms for the distinctive collection of values possessed by each individual.

On the basis of the most reasonable assumptions we can make, we would expect to find in a population of 100,000 well individuals not more than a very few thousand at most whose anatomy, physiology, and biochemistry are "normal," if by normal we mean that their values for one hundred measurable items (an extremely modest number) are *all* within the median 95 per cent range of the very same population (Williams, 1957). If the values for the different items are randomly distributed among the individuals (which may be the case if the one hundred measurements are of diverse character) and if each item yields a Gaussian distribution curve, the number of "normals" in 100,000 would be 592. If the number of measurable items is much larger than one hundred, the number of "normals" approaches the vanishing point. The concept of "normality" as applied to individuals thus takes on an altered meaning and the term "normal" becomes useless.

Averages, standard deviations, and norms *do not exist* with respect to anything that inherently possesses a pattern. Not one of these terms can be applied to one's complete blood pattern; not one can be used in connection with the pattern of a rug or a tapestry. To speak of an average landscape, a normal musical composition, or to say that a particular sunset varies one standard deviation from the mean is talking nonsense because all of these things possess patterns. It is for this very definite reason that it is nonsense to speak of an individual's blood being either average or normal. For the very same reason one cannot speak with discernment of a normal person. The person so designated may have a normal plasma cholesterol value (i.e., one not deviating from an established norm) but if he is at all typical he is not "normal" in every measurable respect. If a physician uses the term normal loosely in talking to his colleagues or patients, this is, to a degree, immaterial. If the researcher who is trying to find out the origins of a disease, thinks in terms of an indefensible concept, it can seriously thwart his efforts.

Importance of Disconformities

The outstanding need in connection specifically with the muscular dystrophy problem is to know precisely what the disease consists of and how it originates. It it generally admitted that hereditary factors are important. In my thinking this is not exceptional; hereditary factors are probably important in determining at least the severity of every individual case of disease regardless of its type. But in muscular dystrophy, as in all other diseases, environmental factors must be at work, too. In order to change these environmental factors effectively in such a way as to remedy the difficulty, it will be necessary to have far more detailed information with respect to the exact nature of the hereditary difficulties. Barring lucky accidents, which cannot be counted on, a better understanding of the disease must come before successful treatment can be devised.

It seems obvious that if we knew all of the detailed differences —anatomical, physiological, and biochemical (including those at the cellular level)—between an individual who is a victim of muscular dystrophy and another individual of the same sex and age who is completely free both from the disease and from any tendency toward it, we would be at least well on the way toward an understanding of what the disease is. This of course is a tremendously large order because there is so much that we do not know about well individuals with respect to their gross and cellular anatomy, physiology, and biochemistry.

On the way toward the objective of understanding the disease it seems desirable that we learn as rapidly as we can about the distinctive features in the macroscopic and microscopic anatomy, physiology, and biochemistry of muscular dystrophy victims, which set them apart from those who do not suffer from the disease. This objective seems an obvious one and is shared, I feel sure, by the great majority of those who are concerned with research in the muscular dystrophy field. The means for approaching this objective expeditiously, however, require clarification. A modification of the concept of normality is one prerequisite; attention to what I will call *disconformity patterns* is another. These two ideas are closely tied together.

The concept of *disconformities* in general is a new one to medical science; neither the word nor a synonym for it occurs in

modern medical dictionaries. Because of preoccupation with the dubious normality idea, disconformities (marked deviations from the average) occurring in the general population have not received attention. These include features of an anatomical, physiological, or biochemical nature which are possessed distinctively by human individuals, both well and ill. Our background for studying the disconformities possessed peculiarly by muscular dystrophy victims is thus exteremely weak. No one has ever made a catalogue or classification of the disconformities which might be studied.

Due to our ignorance of disconformities in general (except that they are often very striking and must be distributed throughout the entire population) it is impossible to formulate a definition with any assurance that it will remain satisfactory in years to come. As a first conservative approximation, however, it seems likely that if an individual consistently yields a value which is in the highest or lowest centile of the general population, this may be looked upon as a significant disconformity. Depending on the particular item under consideration, it may be that values in the upper and lower *quartiles* would constitute significant disconformities.

If the traditional view of normality is accepted, then one must regard any value outside the established norm as *abnormal*, and anything within the established range as *normal*. For example, accepting for purposes of discussion Keys' range of plasma cholesterol values, any individual of age 55 who exhibits a level of 340 mg. per cent is normal in this respect. If the value were 385 mg. per cent, however, the individual would be abnormal. According to the modified view *both* individuals with 340 and 385 mg. per cent cholesterol in the plasma, respectively, exhibit *disconformities*, one slightly more extreme than the other. It is entirely possible that any plasma cholesterol value over 250 mg. per cent in a man of 55 can eventually be interpreted as one sign of possible impending difficulties. If so, this would constitute a significant disconformity.

Clearly in attacking the muscular dystrophy problem one would not center his attention at once on disconformities in eyesight, in skin structure, or in bone composition (though these are often most striking), because they have no obvious connection with the disease. Rather one would be inclined to study discon-

formities (a) in the area of muscle and nerve composition and function, (b) in blood composition, since nourishment for blood and nerves comes from this source, and (c) in urine composition since it is distinctively different for each individual and carries away waste products from muscles and nerves.

No one can tell in advance, however, the precise direction from which light will come, and it would be unfortunate if the areas in which disconformities were sought were too restricted. Do gross disconformities such as in liver size, in spleen size, in adrenal size, or the thickness of the adrenal cortex, for examples, have anything to do with muscular dystrophy? Presumably not, yet in view of our general ignorance in the area of disconformities, it would be dangerous to rule these out. There is wide inter-individual variance in the general population with respect to each of these items.

The exploration of disconformities in general is a challenging project of enormous proportions, and one in which cumulative information gathered in one area can be of great use in another. If knowledge about disconformities were abundant, if they were already explored in the areas of multiple sclerosis, polio, myotonias and in myasthenia gravis, the task of studying them in relation to muscular dystrophy would be enormously simplified. Conversely, when disconformities and their relationship to muscular dystrophy have been explored, investigation in other related fields will be made vastly easier.

Medical science has been familiar since the days of Garrod with "inborn errors of metabolism." These are exhibited by rare cases in which individuals (albinos, alcaptonurics, cystinurics, phenylketonurics, etc.) exhibit faults in their metabolism which are clearly in the abnormal range. Far more significant than these, because they are probably exhibited by everyone in a representative population, are the "inborn disconformities of metabolism." These show up, for example, as high or low (but not necessarily abnormal) urinary levels of specific minerals, amino acids, etc., as disconformities in composition of blood, digestive juices, cerebrospinal fluid, and tissues, as disconformities in enzyme levels in blood and tissues, as disconformities in hormonal activities, etc.

Among the disconformities (possessed by all individuals) which have been explored very little and yet offer much promise

are those in the area of response to the administration of chemicals—both physiological and nonphysiological. Draper, Dupertius, and Caughey as long ago as 1944 called attention to the fact that it is easy to devise tests, even of a pharmacological nature, which may safely be used on human beings. They called attention, however, to the extreme difficulty of interpretation. "All these tests," they say, "have one thing in common, namely marked differences in the pattern of reaction shown by different individuals."

The concept of disconformities should aid in the interpretation of such findings and should stimulate research in this area which is largely untouched. While it is well known that individuals do not react uniformly to drugs and chemicals in general, such diversities are treated as though they were isolated events—not worth recording or studying because they just happened. With a modified view of normality and of disease susceptibility, however, such observations might be the basis for exploring highly meaningful disconformities. If muscular dystrophy patients were found to exhibit a characteristic pattern of responses to a series of drugs, for example, this information might easily lead to a better understanding of the roots of the disease.

The number of disconformities which can be studied (presumably most of them are related to some diseased condition or disease susceptibility) is limited only by the number of kinds of measurements that can be made in the vast areas of anatomy, physiology, biochemistry, and biophysics.

Disconformities are moreover not limited simply to high and low values with respect to the various measurements. Our limited work in this field has revealed that some individuals, for example, may yield unusually *constant* specific blood values when successive samples are drawn for analysis; other individuals show in contrast a high degree of *variability* with respect to the same item (Williams, et al., 1955). That disconformities involving high variability may be significant is indicated by the fact that in schizophrenia, for example, high variability in urinary constitutents has repeatedly been observed (Bellak, 1948; Young et al., 1951).

The usefulness of the concept of disconformities is by no means restricted to the muscular dystrophy field. Because a young man passes a medical examination (for example, for life insurance)

it is by no means safe to conclude that he has in his makeup no roots of disease (Williams, 1957). Ten, twenty, or thirty years later a disease is likely to appear which is related to the disconformities of his own metabolism. A host of diseases of metabolic origin exist, many of them of obscure etiology, and these eventually affect a substantial proportion of the whole population. A study of disconformities during youth will unquestionably uncover the roots of many diseases of later life.

Study of Patterns

While mathematical study of patterns (in the sense we are using the term) is extremely limited, the patterns themselves are of great interest in diagnosis. Diabetes mellitus is characterized by hyperglycemia, glucosuria, polyuria, and acidosis. No one of these symptoms alone is an adequate basis for diagnosis, and each of the diagnostic signs may be accentuated or diminished in any specific case.

The patterns of disconformities possessed by each individual (who may be only temporarily well) should be significant if we know how to interpret them. Handling patterns mathematically becomes a problem because the devices which work for single items—averages, norms, standard deviations, distribution curves, etc., are not applicable. One elementary device which I have had occasion to use is that of determining the "coefficient of resemblance" between two patterns. If in a series of measurements two individuals yield the values indicated in Table 2, a coefficient of resemblance for each item can be determined by dividing the smaller of the two values by the arithmetic mean of the two. By averaging these (to produce .87 in the case of the five items considered) a coefficient can be obtained which will relate the two patterns.

TABLE 2

Coefficient of Resemblance

	Item a	Item b	Item c	Item d	Item e
Individual No. 1	2.0	33.0	1.8	69.0	4.3
Individual No. 2	1.6	45.0	2.2	41.0	4.5
Similarity	.89	.85	.90	.74	.98

It must be admitted that a "coefficient of resemblance" derived in this manner yields far from complete information regarding how the patterns differ, and does not take the place of a more detailed analysis of the variations involving individual items.

It seems obvious that sooner or later the use of high speed computers will come into practice in connection with the exploration of the disconformity patterns possessed by individuals. It is only by their use that extremely large amounts of complicated data can be manipulated in a practical way. Not being an expert in the field of computers I will not discuss the possibilities except to say that computers will be able to calculate coefficients of resemblance (or to make more elegant functional derivations) and to sort the items within the patterns of individuals to be compared, which fall in prescribed categories. It seems likely that the magnitude of each disconformity can be brought into the picture and that there may be, for example, first, second, or third order disconformities. Antecedent to these developments, however, suitable measurements of great variety must be made. Data that are now commonly available are not suitable for this study; only individual data can be used as a basis for studying disconformities and disconformity patterns. Presumably for each disease susceptibility a different pattern of disconformities will be observed.

When the traditional view of normality is accepted by those engaged in etiological research, this means a rejection, as normal, of a large number of potentially significant values derived from laboratory study. This means a tremendous wastage of laboratory time. The use of computers will make useful a large amount of data that would otherwise be without value. A high speed computer will, however, handle data only in the manner in which it is instructed, and unless the disconformity concept is fed into such a machine, the data will never be considered from that point of view. It is my opinion that the disconformity data are the most important data that can be utilized in etiological research and that such data are incomparably more valuable than "abnormality" data based upon traditional ideas of normality.

The collection of sufficient disconformity data so that patterns can be studied and compared will require a modification of the more conventional laboratory approach. Currently it is very common to develop a new laboratory method, for example, and then perhaps on the basis of a hunch, explore its use by applying it to

many individuals. This, of itself, yields little disconformity data. What is required is that many methods (measurements) be applied to the same individuals, because in this way only can their patterns be determined and compared. It is easy to find in the literature the results obtained when a specific measurement has been applied to 100 individuals, but one never finds the results of 100 measurements applied to one specific individual.

Reasons why such data have not been collected are based in part on the extreme specialization of interests of research workers, on the fact that such a large amount of work may be involved, and that undigestible data that no one knows how to use or even publish is likely to pile up. Attention specifically to disconformities and disconformity patterns offers a way out of this difficulty. These can be set down for each individual studied, and the probability that they will be found significant so far as disease susceptibility is concerned is high.

The emphasis on disconformities and disconformity patterns is important from the standpoint of diagnosis of muscular dystrophy patients. Muscular dystrophy is considered to be of several types, perhaps five (Denny-Brown, 1957), based upon differences in the location of the affected muscles, the progress of the disease and the prevalence of different symptoms. The lines of demarcation are not clear-cut; cases exist in which experts cannot agree even on the basic diagnosis. The study of disconformities and disconformity patterns should clear up this confusion with the result that muscular dystrophies may turn out to be a specific number of definable diseases each characterized by its own disconformity pattern.

One of the important outcomes of studying disconformity patterns will be the ability to diagnose conditions early and to institute appropriate prophylactic treatment and care before irreparable damage is done. Mild disconformities may show up early, long before what would now be regarded as abnormalities appear. When abnormalities have arrived, it may be too late to carry out remedial or preventive measures.

Another idea in which disconformity patterns should be very useful is in connection with borderline cases especially in those in middle age or later. My lack of medical background tends to diminish in my thinking the importance of giving a particular condition an unequivocal diagnostic label. The very fact that

some types of recognizable muscular dystrophy attack individuals with varying severity even up to the sixth decade suggests the possibility that many elderly people may have muscular conditions *bordering on* muscular dystrophy but are never so diagnosed because of attention only to abnormalities and lack of insight into the significance of disconformities. It is entirely possible that much can be learned about muscular dystrophy by studying the aging of muscles in so-called "normal" individuals who may be normal in many ways and yet show as they advance in age marked disconformities in the area of muscle functioning.

When it comes to research on remedial agents for the prevention and treatment of muscular dystrophy, the fact that there are several types of disease, cannot be disregarded, because it may easily be that what will be effective in one type may not be useful in another. Suppose for example, that a hypothetical remedial agent is actually highly effective in most cases of one of the less prevalent types of muscular dystrophy and not effective when used with the others. If this agent is tested on a series of unclassified muscular dystrophy cases, it is likely that the favorable results will not appear statistically significant. If the criterion of statstical significance were relied upon, a highly valuable treatment *for certain cases* would be discarded. It is even possible that a series of remedial agents can be found that will be effective respectively for every type of muscular dystrophy and yet each one if tested upon an unclassified assortment of muscular dystrophy cases might be discarded as worthless because it failed to exhibit results of statistical significance.

The principle back of this possibility can be clearly seen if we consider the problem outlined below involving three sets of dice as follows:

Set No. 1: A group of 108 symmetrically made uniform plastic dice. If these are thrown on a table in a random fashion, the total spots showing cannot be less than 108 or more than 648. The most probable value will be 378 (the mean).

Set No. 2: A group of 108 plastic dice are made symmetrically except that they are uniformly slightly loaded with a small steel disk, 18 loaded to make 1 show more often, 18 to make 2 show, 18 to make 3 show, etc. If this group of 108 dice is thrown in a random fashion, the total spots showing will again be not less

than 108 nor more than 648, with 378 as the most probable number.

Set No. 3: A group of 108 dice of which 90 are like those in Set. No. 1 and 18 are a uniform assortment like those in Set No. 2. If this group is thrown in a random fashion, the results will be the same as in the other two cases.

The problem related to these three sets of dice is: Will a strongly magnetized plate on which the dice are thrown influence the way they roll? If we roll each of these three sets of 108 dice on a magnetized plate, we find that *as groups*, they behave essentially alike. For Sets Nos. 1, 2, and 3, we would come to the same conclusion: the magnetic field has no discernible effect on the way the dice roll.

This is, of course, a mixed up and spurious conclusion which can only be set right by paying attention in one way or another to individual dice. If any of those from Set No. 1 are thrown a few times individually, the magnetic field will appear to have no effect (this is in accord with the conclusion which would be reached by extensive trials). In Set No. 2 all the dice, when tested individually, will reveal the determining effect of the magnetic field. Each will, practically always, show the same number. In Set No. 3, one sixth of the dice will be affected and five sixths will not.

This example is, of course, an artificial one and the loading in a human population would never be as uniform as that described. Nonetheless a human population can be loaded in a sufficiently random way, so that a treatment which is unquestionably effective for some individuals can be discarded as worthless if we rely on statistical significance as our criterion.

In this case there seems to be no escape from paying attention to individuals. This to some may seem like an unreasonable and unwarranted expenditure of time; in fact some may regard it even as unscientific since science deals with generalizations which constitute its life blood. No one, however, would advocate the utilization of invalid and spurious generalizations regardless of how valuable they might be if they were true. Only individuals are afflicted with muscular dystrophy. It is not a group disease, and if we hope to find its roots we will have to look into individuals, not into groups.

Cellular Nutrition

Because my background in nutrition is built upon experience in dealing with determining the requirements of yeast cells, I am less inclined than some to neglect the undeniable and inescapable fact that nutrition involves not merely feeding the body as a whole, but also includes the proper nourishment of the many kinds of cells that make up our bodies. When differentiation takes place, the cells eventually produced not only differ tremendously in size, form, and composition, but also in their nutritional needs. Recent tissue culture studies have demonstrated, for example, that certain liver and intestinal cells require nutritionally arginine, cystine, glutamine, and histidine, none of which is regarded as essential for the body as a whole (Eagle et al., 1957). It must be that certain other cells in the body concontaining different assortments of functioning enzymes are capable of producing these amino acids and passing them on to other cells that need them. This observation, if completely valid and correctly interpreted, has tremendous implications for understanding how nutrition works.

Another fact of tremendous import, which I personally came to appreciate through my experiences in the realm of yeast nutrition, is that nutrition is extremely flexible in that cells can be nourished at many different levels of excellence. Yeast cells, for example, can, in a medium containing only salts and very slightly impure sugar, propagate and appear to thrive and be morphologically perfect. Yet by improving the nutrition *in many ways*, they can be made to produce a thousand or a million times as many yeast cells in the same length of time.

I can only suppose that the same principle holds for the cells in our bodies. Their nutrition does not involve merely the getting or the failure to get food but all the different types of cells are capable of being nourished at many levels of excellence. Even cells which appear morphologically perfect may have their physiological functioning vastly enhanced by improving their nutrition. Cellular eutrophia is by no means an all-or-none phenomenon. A multitude of degrees of eutrophia must exist.

Related to this idea is the probability that eutrophia tends to diminish with age. While we have inadequate insight into the process of aging, we do know that cells must lose their physiologi-

cal vigor and this is probably tied in with their waning ability to obtain the necessary nourishment.

Of all the environmental influences that affect living cells, no other (excluding poisons and infectious agents) can remotely compare in importance and versatility with nutrition. Oxygen, water, and a suitable ambient temperature are all absolutely essential, but there is only one kind of oxygen, one kind of water, and one kind of temperature needed. These needs vary in quantity but not in quality. Nutrition is also absolutely necessary, but it covers a multitude of factors and nutrition can vary tremendously in quality as well as quantity.

Faulty cellular nutrition from the quantitative standpoint is involved in every disease where blood circulation is inadequate. Lack of sufficient blood supply can cause severe disease in any organ or tissue, and cellular malnutrition in the quantitative sense, is a highly important factor. The saying that "a man is as old as his arteries" emphasizes the importance of maintenance quantitatively of adequate cellular nutrition throughout the body. Nourishment of nervous tissue is crucially important, and when the blood supply to the brain is too limited, many types of pathology, including death, can ensue. It is worthy of note in this connection that lack of exercise is damaging to muscular dystrophy patients. The basis for this may be in the fact that blood circulation and nutrition of the muscle cells in the quantitative sense is impaired by inactivity.

The quality of cellular nutrition must also be of utmost importance, but this is an area where little definitive knowledge exists and for the time being we must be content with intelligent surmises. Clearly since different cells and tissues have different functions and carry out different biochemical transformations, it follows that the quality of the nutrition varies from tissue to tissue.

It is interesting that the term muscular dystrophy means etymologically "muscular malnourishment." The fact that muscle cells and tissues waste away in this disease no doubt suggested that they do so because they cease to accept nourishment. While this idea is a most plausible one, we need to know, if we are to undertake remedial or prophylactic measures, why and in what sense they cease to accept nourishment.

Dystrophic muscle cells if they are going to be influenced in the

direction of better health, must of necessity be influenced environmentally. Of all the environmental influences over which we have control, certainly nutrition offers by far the greatest number of possibilities. We may presume by now that the disease is not essentially one involving infection, an external toxic agent, anoxia, anhydria, or a faulty temperature regulation and that it cannot be remedied by measures appropriate to these potential causes. The one outstanding environmental possibility remaining is that of nutrition. While no one can categorically deny the possibility, the probability appears remote that any magic non-physiological chemical will be found that can make well the ailing muscle cells which characterize this disease.

Can it be that for some reason which we do not understand (possible akin to the process of aging) muscle cells in this disease fail to be well and thriving and fail to accept the nutrition furnished them by the blood because their nutritional demands have been altered? Can it be that the blood is faulty and fails to deliver the proper nutrients in a suitable balance? If the answer to these questions is no, the possibility of finding effective remedial or preventive measures appears remote. If the answer is yes, there is obviously hope that this alteration can be discovered and that ways can be devised for furnishing the ailing cells what they need, perhaps even before overt disease appears.

Because I take the optimistic view, I strongly advocate learning more about cellular nutrition in the hopes that the secret of remedial or preventive action lies in this direction. If it doesn't lie in this direction, where in the absence of causative infective or poisoning agents, can it lie?

The outstanding work of Beadle and Tatum which has recently received recognition by the award of the Nobel Prize, clearly brings together for the first time genetics and nutrition. Organisms which are genetically different would be expected to have nutritional needs that differ, quantitatively at least, because the enzymic equipment is distinctive for each. Organisms which are genetically identical (if there are such) must of necessity have identical nutritional needs.

From these facts it follows that the nutritional needs of the muscle of each genetically distinct individual must be distinct and different to a degree. Individuals who are prone to become muscular dystrophy victims are no exception to this rule, and it seems

reasonable to suppose that the pattern of these needs is similar for all potential victims. It seems reasonable to postulate further that progressive muscular dystrophy becomes *progressively* worse with time because cellular nutrition which is not geared to needs of the individual's muscle cells tends to weaken these cells and makes them even less able to accept and utilize the not-wholly-satisfactory nourishment that is furnished them by the blood.

This hypothesis has two tremendously important advantages. First it seems to be almost the only one which offers hope. Second, it is to date almost totally unexplored. The nutritional experimentation which has so far been carried out in connection with muscular dystrophy has been for the most part naive, fragmentary, and unsophisticated. It has not recognized adequately (a) that nutrition is only as strong as its weakest link, (b) that cellular nutrition is not identical to the nutrition of the organism as a whole, and (c) that a wide assortment of nutritional factors received *together* in proper proportions must be involved.

To illustrate these three points I will make the following comments: (a) While rabbits suffer from a muscular dystrophy-like disease when deficient in Vitamin E, it does not follow at all that this would be the weak link in human muscular dystrophy patients. We might reasonably take this as evidence, however, that *some* nutritional link (or links) is probably involved. (b) It can easily be true that some specific mineral, some specific amino acid (which need not be one of the "essential" ones), or some one or more of the numerous vitamins fails to get to the muscles in adequate amounts not because it is lacking in the diet of the individual, but because it fails for some reason to arrive at the desired destination.[b] The list of nutritional factors involved in the proper nourishment of muscles must be very large. It may include all the minerals (including trace minerals), all the amino acids, all the vitamins. It may also include a number of metabolic substances which muscle cells need but which are not needed by the body as a whole.

I should like to see a few muscular dystrophy patients treated intensively by nutritional means, bearing all these facts in mind. There is, I believe, a strong basis for hope that the disease can be arrested by this means and that when knowledge in the area of cellular nutrition becomes more advanced, the disease can be prevented. If careful measurements should show no improve-

ment after comprehensive nutritional trials, I would be very much surprised. If there were some definite improvement—even a little—I would take this as evidence that the nutritional attack is an encouraging one and that further nutritional trials, which are of necessity complicated, should be carried forward.

In concluding this discussion I want first to confess that it is incomplete. It is intended to be suggestive rather than definitive. I have left unsaid many things that are pertinent and worthy of more adequate treatment. Second, it should be clear that I have not depicted an easy road to a quick solution. The study of disconformity patterns is bound to involve many extensive and intensive investigations. It is my opinion, however, that approaching problems with our eyes open to their tremendous complexities is much more likely to yield favorable results than if we shut our eyes to some of the facts. Thirdly, it has not been my intention to present "the one and only" approach to muscular dystrophy or to etiological research in general. My ideas are presented in the hope that they will be provocative and useful to the numerous investigators who must follow their own inclinations and make progress on the basis of following their own individual judgments. Fourthly, in connection with muscular dystrophy I would not minimize the importance of fundamental research designed to increase our understanding of how muscles and nerves function and are maintained. This basic work is essential and may conceivably contribute in the end more than any immediate practical trials carried out on the basis of our present too meager knowledge.

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* Much of the discussion is related specifically to the problem of muscular dystrophy, but this is somewhat incidental to the presentation of principles which are believed to be widely applicable to many diseases.

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[b] Since this sentence was written, I have been furnished newspaper reports of experiments (from The University of Texas Medical School, Galveston) which involved joining muscular dystrophic mice parabiotically with mice free from the disease. The results tend to support the concept outlined.

SPLENIC REACTIONS IN ASPHYXIATION

C. DE CHENAR*

This report represents a part of a study on patterns of reaction of the vegetative nervous system and of selected viscera in cases of hypoxemia, organ or tissue anoxia, as well as generalized asphyxia, with particular reference to vasomotor phenomena. For the last decade, through access to non-institutional medico-legal material, in addition to institutional cases of suffocation due to laryngo-tracheal occlusion by aspirated food, observations have been made, mainly on organs with abundant blood supply. The anatomical and histological structure of the organs was compared with corresponding findings of cases of similar somatic constitution, but without asphyxiation.

From the earliest cases on, observation accumulated concerning changes in the spleen which appear to be unmentioned in text and reference books, as well as in the available literature. Recent texts on forensic medicine (1) make reference to the work of Reuter (8), published in 1903, for information on anatomical changes in the spleen, attributed to asphyxiation. Reuter summarizes macroscopical observations on the spleen of 41 cases of asphyxiation. In the majority of his cases, the capsule of the spleen is corrugated and the volume decreased, but in others, a certain degree of congestion occurs. No histological studies are included. He quotes the only existing reference of his time, Szabinsky (1865) who collected similar observations, in part, in animal experiments. In conclusion, ischemia and some hyperemia are described.

The changes to be recounted here, center around hemorrhages in the spleen, besides irregularly scattered areas of ischemia or passive congestion, visible in microscopic preparations only. These hemorrhages occurred in every case of suffocation, drowning, strangulation, asphyxiation due to laryngo-tracheal occlusion and sudden death with anoxemia. They occur in embolic occlusion of the coronary arteries and, fatalities of electro-convulsive therapy. In the majority of cases, where information was available, attempts were made to support the splenic changes and to explain those with the eventually known occurrence of a

hemorrhagic purpura, vascular, coagulational or mixed type. In no case were the latter conditions present, therefore, the hemorrhages observed in the spleen could not have resulted from those.

The condition of the spleen in cases of death from other causes —which might serve as normals,—as it is found in daily routine work, could be summarized in two groups:

(A) In a large percentage of cases of chronic diseases with gradual deterioration, there is no deviation from the histological standard structure of the spleen. In others, there might be an irregular distribution of mild congestion.

(B) In cases of rapidly progressing deterioration, including terminal cardiac decompensation, various degrees of passive hyperemia predominate, mainly in the red pulp, interrupted by extensive areas of ischemia which frequently show a nearly complete absence of cellular elements of the circulating blood. In the more than 900 cases included in this study, there were no identifiable hemorrhages, unless the final period of the moribund condition met the criteria of sudden death with evidence of acute anoxia as, for example, in the event of thromboembolic occlusion of the coronary arteries.

In each case of the splenic hemorrhages, there are differences in their location, dimensions and intensity, as compared with other cases. The borderlines of the hemorrhages, however, are similar so far that there is no reaction of demarcation around them. This indicates acutely developed, pre-mortal changes.

Depending upon the immediate cause and mechanism of death and, presumably upon individual factors in the chemo-receptor-vasomotor reflex chain, the following types of localization and extent of hemorrhages occurred in the cases included in this study:

(1) Capsular and subcapsular. In the first case, there is infiltration of the deeper layers of the capsule by erythrocytes with eventually continuous hemorrhages in the underlying red pulp. The subcapsular hemorrhages are scattered or diffuse, often severe, invading the entire splenic substance, both red and white pulp. (Fig. 1)

(2) Trabecular. Deep vertical extensions of otherwise superficial hemorrhagic areas, partially confluent, partially sharply outlined or showing gradual transitions toward the surrounding,

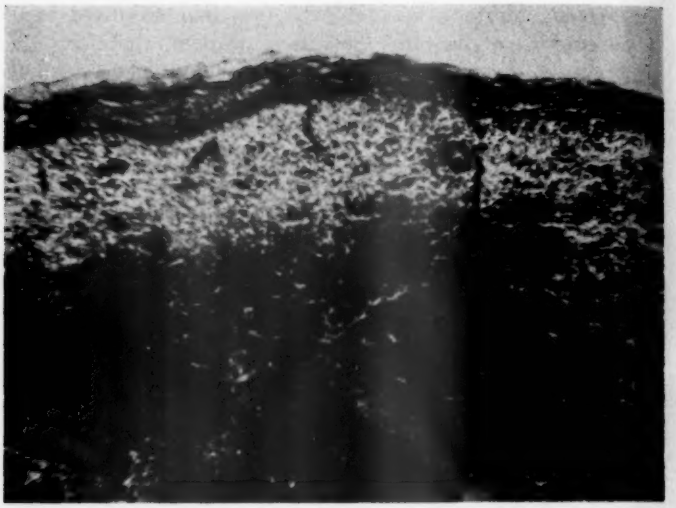

FIG. 1. Massive subcapsular hemorrhage, involving the entire superficial layer, with only narrow intermediate areas unaffected. Asphyxiation due to strangulation. Turnbull's blue reaction after previous mordanting with Perl's solution. 120x.

unaffected, structure. These hemorrhages originate from ruptured intratrabecular arteries. The dimensions of the resulting hemorrhage vary considerably, regardless of the diameter of the artery or trabecula involved. (Fig. 2)

(3) Diffuse, destructive. These hemorrhages originate from trabecular, central or smaller arteries. They obliterate the splenic structure to such an extent that no parts can be recognized but a few trabecular fragments of the thinner branches, while some heavier trabeculae might be preserved. (Fig. 3)

(4) Generalized petechiae. This type of splenic reaction is different from the previous groups. It occurs in cases of lethally ending electroconvulsions. In those studied here, death ensued in less than half an hour, in spite of the most adequate and competent intervention to avert it. In the entire spleen, irregularly scattered, small hemorrhages develop. Sections of the central nervous system show characteristic stigmata of the most severe angiodynamic collapse, areas of complete ischemia interchanging with those of intraparenchymal hemorrhages assuming dimen-

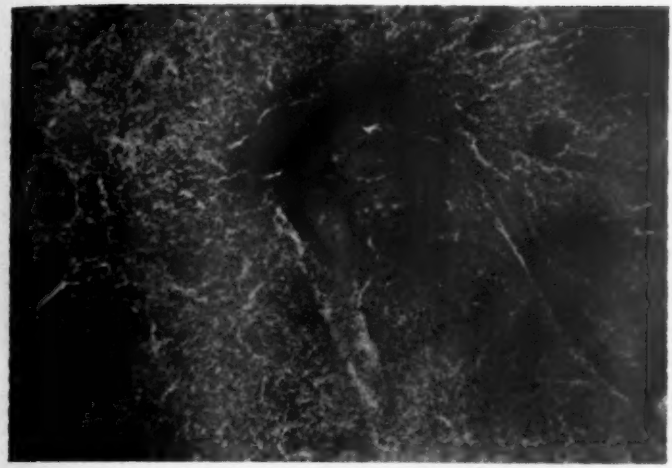

FIG. 2. Peritrabecular hemorrhage, infiltrating the white pulp, with less involvement of the red pulp. Asphyxiation in accumulated CO_2. Turnbull's blue reaction, 120x.

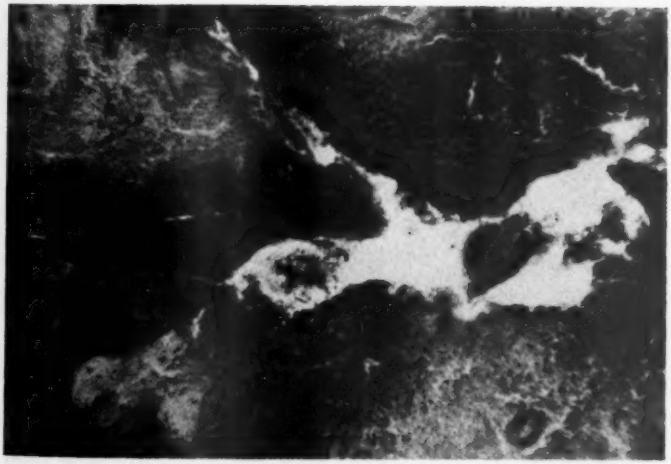

FIG. 3. Diffuse hemorrhage, as the result of total destruction of a medium sized trabeculla. Asphyxiation in propane-butane gas. Turnbull's blue reaction for hemoglobin, 120x.

sions up to macroscopic visibility, in the medulla oblongata. (Fig. 4)

Successive stages in the development of hemorrhages are accessible to study in most cases. Proximally from the point of rupture, there is maximal contraction in the blood vessels, with no visible contents. But in the opposite direction, there is a massive accumulation of cellular elements and blood plasma, all expanding into the surrounding structures. The transition of hemorrhagic areas toward intact fields, or, the delimitation of the two, varies in the different types observed. In most cases, the outlines are sharp. In others, the hemorrhages infiltrate their surroundings, as in most cases of slower developing anoxia.

Comment

From the specific viewpoint of this study, the spleen is considered only with reference to its function as a blood depôt. In the capsular—trabecular framework of the human spleen, in addition to elastic elements, there is a limited proportion of smooth muscle fibers. These are under direct neural control, via diencephalon—medullary centers—celiac plexus (9). Along the arborization of splenic arteries, non-myelinated fibers extend

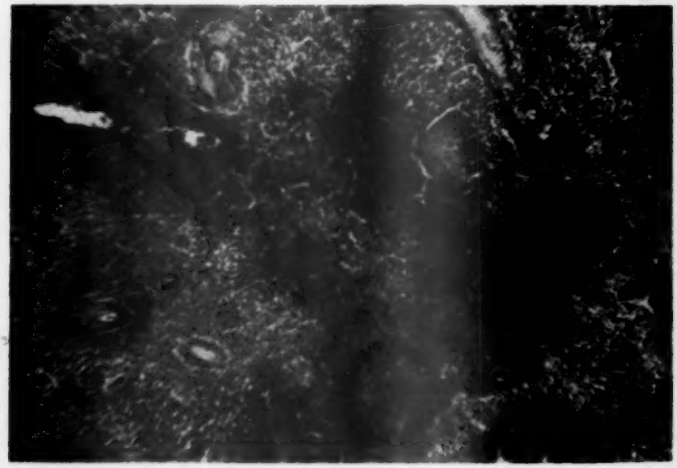

FIG. 4. Irregularly scattered hemorrhages in both white and red pulp. Exitus post E.C.T. Turnbull's blue reaction for hemoglobin, 120x.

down to the level of the sinusoids. Even the spiral, parietal, elements of the sinusoids, at their transition into the non-muscular venous sinuses are, presumably, under neural control (9). Denervation and sufficiently long survival of the spleen is followed by maximal dilatation and atrophy of the contractile elements (3). The non-contractile, fibrillary-syncytial wall of the sinusoids is, as a rule, non-permeable for corpuscular elements (10). The red pulp, on an anatomical and physiological basis, might be subdivided as follows: (a) subcapsular, (b) perifollicular and (c) interfollicular sections. Carbon particles are deposited in the perifollicular area (2).

Functionally, the spleen is a depôt, mainly for erythrocytes, within the portal system. The volume of the spleen, as a reservoir, is regulated by changes in the intensity of sympathetic and parasympathetic impulses, as in the case of blood vessels. The smooth muscle fibers of the capsule and of the trabeculae increase the dimensions of volume control by the splenic blood vessels (7, 10).

Rotter and Büngeler (9), with references as given, outline the regulatory function of the spleen by dividing the action of the components in two ways. First, regular "systolic-diastolic movements", in accordance with the demands of the portal hemodynamic equilibrium. An increased hepatic resistance results in accelerated contractions (6). Elevated vagotonus causes increased hepatic circulatory resistance against portal passage (9). Second, "singular contractions" of maximal intensity of all contractile elements in cases of acute, absolute or relative, oxygen deficiency, in order to inject all available erythrocytes into the general circulation (9). As an agonal sign, some erythrocytes may appear in the mesh of the pulp (4, 5).

The material substratum for the phenomena described in this study might be found in the second, "emergency function" of the spleen. The singular contractions of all out force, in emergency situations, as found in every case in this study, are oversized in amplitude against the limit of resistance of the contractile and elastic structures. Therefore, they lead to the disruption of the contractile and elastic elements, causing the hemorrhages which, in this way, constitute a splenic sign of asphyxiation.

This study includes more than 50 cases of asphyxiation, as

tracheo-bronchial occlusion, strangulation, drowning, accidents and suicides with CO, CO_2, propane-butane gas and Cl fumes; furthermore, sudden deaths due to acute cardiovascular circulatory failure. Splenic hemorrhages occurred in every case, and they were different from areas of congestion and ischemia attributed to agonal conditions in other cases.

Summary

Hemorrhages of various location, extent and delimitation are described as the result of a microscopic study of the spleen in cases of fatal anoxemia and asphyxiation. A classification of the types so far observed is given, as well as an interpretation of the phenomena on borderline physiological basis, as the resultant of a singular, maximal, contraction of the smooth muscle elements of the splenic structure in an extreme emergency, in order to add all the erythrocytes available to the circulating blood. The force of this contraction is postulated to exceed the limit of resistance of the capsular—trabecular—vascular framework, with the resulting hemerrohages here described. These hemorrhages constitute a splenic sign of asphyxiation.

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THE AFFINITY OF HEPARIN FOR CERTAIN LIBERATORS OF HISTAMINE

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Heparin and histamine coexist in mast cells (Holmgren and Wilander, 1937; Riley and West, 1952), and it has been postulated by Paton (1955, 1957) that there exists between the two an acid-base attraction such that histamine can be displaced from this binding by substances with stronger base properties. The salient point of this "ion exchange" theory of histamine release is an affinity between the heparin and the histamine releaser. *In vivo* release of histamine is accomplished by a wide variety of substances including many organic bases (MacIntosh and Paton, 1949; Paton, 1955).

The ability of certain basic substances to release histamine has been correlated by MacIntosh (1955) with their affinity for heparin. A similar comparison has been made by Paton (1955) of a larger number of substances with respect to their ability to form a precipitate with ribose-nucleic acid.

This report consists of a quantitation of the affinity of heparin for many histamine liberators as measured by: 1) their power to dispel the metachromasia of a heparin-tolouidine blue complex, 2) the ability of certain of these liberators to inhibit heparin-activated lipemia-clearing factor, and 3) heparin's effect on the toxicity of two histamine liberators.

Materials and Methods

To quantitate the dispelling of metachromasia by a histamine liberator, the method of MacIntosh (1941) for the assay of heparin was utilized with the substitution of .05 M phosphate buffer (pH maintained at 7.3–7.4) for the NaCl solution as a diluent. In this procedure, the substances to be tested for heparin affinity were added to an aqueous heparin-toluidine blue mixture containing 50 micrograms of each. Interference with the heparin-toluidine blue complex increased the amount of uncombined toluidine blue thus increasing the blueness of the aqueous phase. The transmission of this aqueous solution was read in a Coleman-Junior spectrophotometer at 660 millimicrons, or in a Klett-Summerson photoelectric colorimeter with a No. 66 red filter.

Under the conditions of this test, a certain amount of toluidine blue remained in the control tubes after the extraction step, and this amount was

subtracted from the toluidine blue in the experimental tubes when their concentrations were read from a standard curve. This standard curve was prepared for each set of determinations by plotting the colorimeter readings against decreasing amounts of dye diluted in buffer to the same total volume as used in the test.

The effect of histamine liberators on heparin-activated lipoprotein lipase was studied as follows.

Two groups of Sprague-Dawley Holtzman rats were used. One group with an alimentary lipemia supplied lipemic plasma. The other group, injected intraperitoneally with heparin 1 mg/kg, served as the source of clearing factor. Blood was taken by cardiac puncture and the plasma from each group of rats was pooled. Lipemic plasma, plasma containing clearing factor, and saline solution of histamine liberators were mixed in equal proportions and incubated at 37° in a water bath. Optical density was read at intervals in a Klett-Summerson photoelectric colorimeter with a No. 42 blue filter. Since heparin is a necessary co-factor to the clearing enzyme (lipoprotein lipase), a lack of clearing indicates interference with heparin action by the histamine liberator. Protamine, a known antagonist of heparin and of clearing (Brown, 1952), was tested to give a base for comparison.

The effect of heparin on the toxicity of tubocurarine and strychnine was studied in white mice of the C57BL strain. Fasted adult male and female mice were injected with an LD 50 of tubocurarine ($0.55 \pm .05$ mg/kg) or strychnine (2.3 ± 0.3 mg/kg) intraperitoneally plus heparin in an amount five times that of the tubocurarine or strychnine. Control groups received only the drug under test.

All drugs used in this work were dissolved in 0.9% sodium chloride solution. They were as follows: medicinal powdered peptone (Stearns); para-methoxyphenylethylmethylamine, "compound 48/80" (Wellcome Research); dextran, 6% in saline (Abbott); atropine sulfate (Merck); strychnine sulfate (Eimer and Amend); stilbamidine di-isethionate (Merck); trimethaphan camphorsulfonate (Hoffman-LaRoche); tubocurarine chloride (Squibb); quinine hydrochloride (Merck); amphetamine sulfate, racemic (Merck); protamine sulfate (Wyeth); heparin sodium (Abbott); polyvinyl pyrrolidone (Schenley); cinchonine sulfate (Nutritional Biochemical); colchicine (Fisher); reserpine (Ciba); papavarine HCl (Merck); histamine phosphate (Nutritional Biochemical); toluidine blue-O (National Aniline); reagent grade acetone (Mallinckrodt); benzene (Baker); and commercial grade mineral oil.

Morphine and codeine sulfates were also used, but the manufacturers' names were not available.

Results

Experiment I

Most of the drugs used in this experiment have therapeutic use and were tested because they are known to be liberators of histamine in man (Paton, 1955). Table I shows the comparative strength of these substances in respect to their ability to dispel the metachromasia of the toluidine blue-heparin complex.

Compound 48/80, known to be a strong histamine liberator, showed much the strongest affinity for heparin of any substance tested.

Polyvinyl pyrrolidone has been used as a plasma expander. It was tested because it is known to produce an anaphylactoid reaction in the dog (Halpern and Briot, 1953). This substance showed a strong affinity for heparin.

Tubocurarine, stilbamidine, and trimethaphan camphorsulfonate, which in therapeutic use often produce symptoms of histamine release (Paton, 1955), showed about equal affinities for heparin.

Peptone was tested because it is said to cause damage to mast cells (Wilander, 1939) and because it is generally accepted as a releaser of histamine. Its affinity for heparin was slight.

The affinity of reserpine for heparin was tested because of two indications that it may be a liberator of histamine, i.e., stimulation of gastric secretion (Barrett, et al., 1954) and liberation of 5-hydroxytryptamine that is located in cells known also to contain histamine (Benditt, 1955; Benditt and Wong, 1957). It should be noted that, since reserpine tends to precipitate from an aqueous solution containing inorganic salts, these extractions were done in distilled water and must be considered in that light.

When first mixed with the heparin-toluidine blue complex, papavarine gave the deep blue color indicative of dye displacement. This disappeared during the extraction step, however, and it was felt that this measurement was too low to reflect the true affinity of papavarine for heparin.

Mescaline was tested because of its striking chemical similarity to the unit of polymerization of compound 48/80 and to one constituent group of reserpine. However, repeated testing showed no affinity for heparin.

Cinchonine differs from quinine, a known histamine liberator, only in having one less methoxy group. It showed an affinity for heparin consistently less than that of quinine.

Because of its powerful effects on mast cells (Padawer, et al., 1956) and the similarities of its chemical structure to reserpine, it seemed likely that colchicine would have some heparin affinity. This proved to be true.

The heparin affinity of strychnine, atropine, amphetamine, morphine, and codeine was small and inconsistent in these tests.

Dextran, which produces anaphylactoid symptoms and histamine release in the rat (Vorhees, et al., 1951), showed no affinity for heparin in these tests.

There are reports that an *in vitro* complexing of heparin and histamine have been shown (Sanyal and West, 1956), but this complexing could not be demonstrated by this method.

Experiment II

The effect of certain histamine liberators on lipemia-clearing was used as a test of the heparin affinity of the liberator under more physiological conditions. From the results given in Table II, it can be seen that compound 48/80 is equal to protamine in inhibiting clearing. Of the other drugs tested, only stilbamidine showed any tendency to inhibit clearing. All other drugs, whether or not they showed heparin affinity by dispelling the metachromasia of toluidine blue, showed no ability to inhibit clearing factor. These results are interpreted as meaning that plasma con-

TABLE 1

Micrograms of toluidine blue displaced by various histamine liberators

Drug used	Micrograms of drug used	Micrograms of toluidine blue displaced
Compound 48/80	100	34.0
Tubocurarine chloride	1000	17.5
Trimethaphan camphorsulfonate	1000	18.0
Stilbamidine di-isethionate	1000	16.0
Quinine hydrochloride	1000	13.0
Strychnine sulfate	1000	0.5
Atropine sulfate	1000	2.0
Amphetamine sulfate	1000	0.0
Morphine sulfate	1000	0.0
Codeine sulfate	1000	1.0
Dextran	1000	0.0
Polyvinyl pyrrolidone	1000	31.0
Peptone	1000	6.9
Mescaline sulfate	1000	0.0
Cinchonine sulfate	1000	11.9
Colchicine	1000	12.6
Histamine phosphate	1000	0.0
Reserpine	1000	34.1
Papavarine hydrochloride	1000	2.8

TABLE 2

Decrease in optical density of lipemic plasma in presence of histamine-liberating drugs

	Decrease from time zero		
	After 15 min.	After 30 min.	After 60 min.
Tubocurarine chloride 0.250 mg/ml	.017	.026	.036
Quinine hydrochloride 0.250 mg/ml	.016	.027	.035
Trimethaphan camphorsulfonate 0.250 mg/ml	.018	.028	.037
Strychnine sulfate 0.25	.015	.029	.038
Morphine sulfate 0.25 mg/ml	.018	.030	.039
Atropine sulfate 0.25 mg/ml	.019	.029	.038
Amphetamine sulfate 0.25 mg/ml	.018	.029	.037
Stilbamidine di-isethionate 0.250 mg/ml	.008	.012	.017
Compound 48/80 0.025 mg/ml	.002	.002	.004
Protamine sulfate 0.025 mg/ml	.002	.004	.005
Control group	.018	.028	.036

stituents can reduce the effect a liberator drug has upon the heparin co-factor of the clearing enzyme.

Experiment III

It was seen from Experiment I that two highly toxic materials, tubocurarine and strychnine, differed greatly with respect to their affinity for heparin. Experiment III was designed to test whether this difference persisted in the living mouse and also whether the complexing wth heparin would affect the toxicity of the drug. Table III shows that heparin, given with tubocurarine in a ratio of 5:1, reduced the LD 50 of tubocurarine to an LD 20. Given with strychnine in a 5:1 ratio, heparin did not affect the toxicity of an LD 50 of strychnine. The effect of heparin on either drug was unchanged whether it was mixed with the drug, given in separate injections at the same time, or given

60–75 minutes previously. These results tend to confirm and extend the findings of Higginbotham and Dougherty (1956) regarding the possible role of heparin in detoxification.

Discussion

Paton, in discussing the possible binding of histamine by tissue acids and their release by organic bases, implied that most basic histamine liberators have at least enough affinity for heparin to displace histamine. He presents evidence to the effect that ribosenucleic acid possesses an affinity for many of the substances tested for heparin affinity in the present work. The criterion Paton uses to judge the affinity of RNA for the histamine liberators is the production of a visible precipitate. The concentrations needed to produce this precipitate are so great (at least in the case of morphine and amphetamine) that a concept of these two substances displacing histamine through a competitive affinity seems highly questionable. One impetus to this present work was the desire to gather evidence as to which of several histamine liberators could likely be operating as such by complexing with heparin. Judged only on the basis of displacement of toluidine blue, it seems possible that several of the substances tested might have sufficient heparin affinity to release histamine by a matter of simple displacement. It also seems equally probable that several others cannot be operating through this mechanism.

TABLE 3

Effect of heparin on the toxicity of tubocurarine and strychnine in C57BL mice

Group 1	0.55 mg/kg tubocurarine chloride	35 dead — 73 total
Group 2	0.55 mg/kg tubocurarine chloride + 2.85 mg/kg heparin	11 dead — 52 total
Group 3	2.3 strychnine sulfate	47 dead — 70 total
Group 4	2.3 mg/kg strychnine sulfate + 11.5 mg/kg heparin	49 dead — 69 total

If histamine release is to occur by "ionic" displacement from tissue acids which supposedly are binding it, the concentration of the releaser at the cellular or subcellular levels is critical. If the releaser enters the organism by the intravenous route, simple dilution will reduce its concentration at the sites of histamine binding to extremely low levels. In addition, it seems clear from Experiment II that even such substances as are present in plasma can reduce the effect which many histamine releasers may have on heparin.

The fact remains, however, that some releaser substances in low doses are quite powerful liberators of histamine. If histamine were bound by loose attraction to many types of compounds other than tissue acids, the effectiveness of small amounts of liberators could be explained by a postulation that the liberator displaced histamine wherever a weak combination existed. It might also be theorized that the liberator was activating histidine disjunction and decarboxylation at innumerable and widespread peptide linkages in a manner suggested by Ungar's work (Ungar and Damgaard, 1954).

High concentrations of histamine often occur where there is a high concentration of heparin, e. g., in mast cells. If much histamine is released from these cells by displacement, then a selective concentration of the releasing substance in these cells must be postulated. In which case, the mechanism underlying the uptake of the releaser is likely to be more complex than an affinity for heparin and must certainly be considered the basic factor leading to histamine release.

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THE STUDY OF PICA AND FOOD PREFERENCES IN A POST PARTUM GENERAL HOSPITAL POPULATION

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The prevalence of earth eating has intrigued many physicians throughout the centuries. Peculiar dietary desires in pregnant women have also been an attractive realm of speculation as well as a source of entertainment or even embarrassment. Pica has received rather little attention in recent years since all attempts to unravel its etiology have been singularly unsuccessful in the majority of cases. The most complete survey of the subject may be found in the current monograph by Marcia Cooper (1).

In 1950 Ferguson and Keaton (2) published an interesting report concerning the ingestion of clay and laundry starch in a rural antepartum clinic population in Mississippi. They reported a clay in consumption in 25% of their cases and a starch consumption in 39%. After carefully questioning their subjects they concluded that the individuals ate clay and cornstarch simply because they liked it. A more recent study by Edwards et al. (3) on the dietary factors involved in clay and cornstarch eating tended to support previous findings but also suggested that particular dietary disturbances were related to ingestion of these particular materials. The present study was conducted to determine if there were differences in a metropolitan post partum population in Texas in comparison with other population studies. Since the population found in this general hospital encompasses a higher income group than the rural Mississippi study, presumably a higher education level, and a more adequate dietary intake; it was believed that there would be significant differences.

Procedures

One hundred random post partum females were questioned using a short form questionnaire and an interview to develop pertinent information. The interviewers were two Southwestern Medical School students, who in some instances, attended these women in delivery and therefore, had a high degree of empathy with the patients and were able to conduct a comfortable and informal interview session. These patients reflect a fairly typical sample of the hospital patient population of the Parkland Memorial Hospital, a City-

County Hospital serving Dallas and the surrounding Dallas County. The random sampling of patients during this six weeks of interview yielded white, Latin American and Negro patients in approximately the normal proportion seen in the Parkland Memorial Hospital situation. In the interview the patients were questioned as to their socio-economic status, particular food preferences during pregnancy, the basis of these preferences, the kinds of foods ingested, the duration of these preferences, and their current physical and nutritional status. The age range of the patients was from 15 to 40 with a mean age of 24 years. Eighty of the patients were Negro, thirteen were white and seven of Latin American extraction. Seventy per cent of the total population were married, 11% were single, 13% were separated and 6% of the total population were divorced. With regard to childbearing, the population ranged from approximately 10% primiparas to one patient having 13 children. The mean number of children for the population was 3.6. The income ranged from nothing to $125.00 per week with the mean income of $35.00 per week.

Results

In reviewing the dietary habits of this population it was found that 48% of the group showed particular food preferences during pregnancy. These covered an extremely wide range of food materials ranging from the frozen condensation of refrigerators to the usual preferences of fruits, meats and desserts. Seven per cent of the population had preference for starch and 4% had a preference for baking powder during pregnancy. On the basis of only a brief review, the medical history and records of the patients indicated that 96% of the patients had good nutritional status.

In the population studied it was found that only the Negro population were clay eaters. The number of clay eaters reflected approximately 18% of the total group studied, however, this population of clay eaters represented 28.5% of the Negro group. All of the clay eaters had families or friends who ate clay. Seventy-six per cent of the group who ate clay began eating clay in the second trimester of pregnancy but all of them discontinued this practice after delivery. Eighty-five per cent of the multiparas had eaten clay during previous pregnancies. Most of the group said that they ate clay because of a "craving" for it but did not know what good it did. A number of them were of the belief that it might cause a tumor but they continued to eat the clay anyway. The use of clay ran from two to three ingestions in pregnancy to the eating of small quantities three or four times a day. Most used the commodity as is, although a few

baked it in an oven, presumably to dry it. It was impossible to determine whether these perverted food habits were encouraged by suggestions from others or whether the individuals noted other people had the same dietary habits after they began the practice themselves. Seemingly, the onset of the habit was quite subtle; the patients consistently debated with themselves frequently changing their minds several times during the interview.

Discussion and Summary

The results of the present study seem to parallel the previous study done in Mississippi, (2) however, apparently far fewer of our patients ate cornstarch during pregnancy. The results suggest that socio-economic status does not significantly alter the tendency to ingest clay during pregnancy when a comparison is made with previous studies. Superstitions and habits regarding clay consumption that were elicited during the interview sessions i.e., marking of the baby, etc., may be found in the historical writing of ancient Europeans as reported in Cooper's monograph (1). The common explanation of "craving" the peculiar items reported leads us to believe that the incentives were psychic and that the ethnological background of the Negro patients leaves them peculiarly vulnerable to primitive imitated habits. It should be noted that no ill effects could be found associated with clay and cornstarch consumption. (3). The present study further presents some of the parameters of food preferences and clay cornstarch ingestion in a population of females in a City-County hospital in Dallas, Texas area. Further investigations of nutritional and physical status of clay eating patients are planned.

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AMYLOIDOSIS AND OTHER PATHOLOGIC CHANGES IN MICE EXPOSED TO CHRONIC STRESS*

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Various apparently unrelated experimental interventions provoke amyloidosis in the mouse (19): indeed, in some strains, the condition is encountered as a spontaneous disease in older individuals (20). Recently we have found that it may be induced in young mice by parabiosis (6). In the rat different lesions, vascular sclerosis and necrosis, also result from a variety of experimental causes (8, 9), are often found in older animals (23), may be induced by parabiosis (7), and also long continued exposure to stress (17). This latter mechanism has often been invoked to account for the spontaneous occurrence of these lesions.

It therefore seemed possible that, in the mouse, amyloidosis might be a somatic sequel to chronic stress, which, if true, would help explain both its prevalence in old individuals of certain strains and its experimental induction in that species by agents of such diverse and varied nature as have been reported efficacious. Inasmuch as we have found long term exposure to electrical shocks to be an effective method of inducing stress (11), and mice to be particularly susceptible (12), it was decided to use this method of testing the hypothesis experimentally.

Materials and Methods

Non-dystrophic mice of strain 129 bred in this laboratory were employed. Older animals were selected with the presumption that they might be particularly sensitive. Thirty-six mice, twenty-four females and twelve males, ranging 9–14 months of age and weighing 18–44 grams were selected. The stressed group, among which deaths were anticipated, consisted of 18 females and 10 males; the control group of six females and two males. All were subjected to unilateral nephrectomy and maintained on a drinking solution of 1% NaCl during the experiment—a procedure known to sensitize animals to certain cardiovascular consequences of stress—and fed Purina laboratory chow.

For eight hours daily the stressed animals were individually housed in cages with electrically activated flooring, a modification of the system recently described (10). In it, they received brief electrical shocks, sufficient to cause them to jump, at two minute intervals. While so housed both they and controls were deprived of food and drinking fluid, freely available at all other times.

The experiment was continued for 71 days, by which time the stressed group was reduced to eight survivors. These, and the same number of controls, were killed with ether and various organs and tissues removed and placed in 10% neutral formalin. When fixed they were blotted dry, dissected free of extraneous tissue, and weighed on a delicate torsion balance, following which they were subjected to histologic preparation and examination. Amyloid and its effects was sought for in the adrenals, liver, spleen, kidney and heart.

Results

As reported previously (12) males proved unable to withstand this type of stress and all died within a week, commonly from respiratory paralysis during a convulsive seizure. The females proved more resistant and, excepting for three which died during the first month, survived for 8 weeks or longer. The body weight declined slightly in both groups during the period of study. Reduced food availability was doubtless contributory, however stressed females had lost 28% of starting body weight as compared to 8% loss in non-stressed controls.

Organ Weights.

Adrenal: Adrenal hypertrophy was conspicuous in the stressed animals, the glands averaging 41% larger than in controls. Some of these glands contained amyloid, but they were not appreciably heavier than those which did not.

Spleen: In the stressed group some spleens were smaller than normal, presumably due to stress-induced lymphatic atrophy; whereas others, with amyloid deposits, were larger. The average splenic weight although greater in the stressed group did not differ significantly from controls, but the standard error of the mean was large in the former because the organs were individually either smaller or larger than in controls.

Heart: In the stressed group the heart was often enlarged and myocardial pathology[b] was observed with greater frequency. As in the case of the kidney, however, enlargement was found only in those mice which, on histologic examination, were also found to have amyloid lesions.

Kidney: The kidneys of stressed animals were larger than those of controls when amyloid deposits were present, otherwise there was no appreciable difference in size. Because so many of the stressed group bore such amyloid infiltrations, the mean renal

weight for the group was significantly higher than in controls. The results are summarized in Table 1.

Pathologic Findings.

All of the stressed males died in the first few days of treatment and thus failed to develop chronic lesions. In the stressed females, however, amyloid deposits in the kidney, spleen, liver, or adrenals, were found in four of the eight which survived the full experimental period and in five of the ten which had died during treatment, but had sustained it for a week or more before death. No amyloid changes were found in any of the controls, in conformity with observations made in other untreated animals of this strain (6).

In all of the affected animals, amyloid was found in the liver and spleen, in most of them the adrenal glands were also affected, and in many the kidneys and hearts were involved.

The characteristic distribution of amyloid in the liver and spleen of mice has adequately been described (20), and in the present material no unique features warranting extensive description were detected. The characteristic appearance is given in figures 1 and 2.

TABLE 1

Sequelae of chronic stress in the strain 129 mouse

	Stressed		Controls	
Data	Males	Females	Males	Females
Initial	11	18	2	6
No. in group Final	0	8	2	6
Age in weeks Initial	57 ± 1.9	54 ± 1.4	54 ± 1.5	50 ± 2.4
	37 ± 1.2	27 ± 1.6	37 ± 2.0	27 ± 1.4
Body weight g Final	.....	23 ± 0.9	27 ± 3.0	26 ± 0.7
Organ weights mg/100g				
Adrenal	.....	41.3 ± 2.0	25.0 ± 6.0	28.9 ± 1.4
Spleen	.....	344 ± 69	205 ± 37	305 ± 26
Kidney	.....	1,766 ± 93	1,614 ± 44	1,434 ± 40
Heart	.....	570 ± 26	538 ± 15	466 ± 18
Amyloidosis	.....	50%*	0	0

* Full term survivors and others equally represented.

In the kidneys the material tended to be deposited in the glomerular capillary tufts (Fig. 3) and, in addition, could sometimes be seen penetrating the interstitial tissue between the tubules. Only in one instance was the renal involvement sufficiently severe to produce necrosis of the papilla which characterizes the more advanced severe cases of murine renal amyloidosis (5).

The most commonly observed deposits in the adrenal glands occurred at the cortico-medullary junction (Fig. 4). In the most

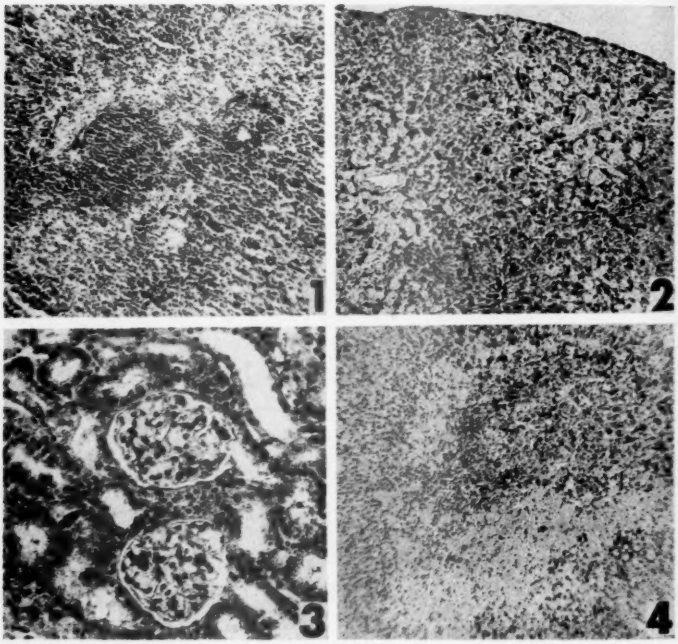

FIG. 1. Amyloid deposits in the spleen of a mouse stressed for 71 days, some of which have a rather typical perifollicular crescentic distribution. H and E x125.

FIG. 2. Diffuse amyloid in the liver of a mouse which died after 5 weeks of stress. Gomori trichrome x125.

FIG. 3. Amyloid deposits in and around glomerular capillaries. Same mouse as in Fig. 2. Tubules are free of the material although some tubular dilation is evident. H and E x320.

FIG. 4. Adrenal section from a mouse stressed for 5 weeks. Amyloid occupies the cortico-medullary junction and extends inward and outward. H and E x83.

advanced cases infiltration of both medulla and cortex was observed.

The adrenal glands of mice exposed to stress, were in general, more hyperemic than those of non-stressed animals, particularly the medulla. Several instances of adrenal medullary hemorrhage were encountered in stressed animals which died during treatment. In some of the survivors, perhaps as the result of a previous hemorrhage, the medulla was replaced by an amorphous but granular material, separated from the cortex by a layer of fibrous tissue (Figs. 5–7). These changes were not observed in control material, and there was no evidence to indicate that they were in any way related to amyloid disease.

The hearts of animals with amyloidosis were, in many cases, found to include areas of myocardial degeneration and replacement fibrosis (Fig. 8). This is a common accompaniment of cardiac amyloidosis in the mouse (20).

It has not always been possible to differentially stain this infiltrative material, although some examples stain metachromatically. Thus it appears to differ chemically from human amyloid, although in its other characteristics it seem identical. This is well recognized in the literature (4, 21) and the hyaline substance is therefore termed "mouse amyloid." The material herein described fulfils the criteria of that substance.

Discussion

All of the animals, stressed or not, lost weight during the experiment, partly perhaps because of restricted access to food. This should have acted as a moderate stress, but did not produce as dramatic adrenal enlargement as that found in animals exposed to electroshock. Despite weight loss, pathologic changes did not occur in the organs of control animals, indicating that this alone was incapable of causing amyloidosis.

Stressed animals were distinguished from controls by having, as a group, larger adrenals, hearts and kidneys. Adrenals were enlarged in all stressed animals, but the hearts and kidneys only in animals suffering amyloidosis, and thus affected 50% of the animals exposed to stress.

Mice appear to be more susceptible to amyloid disease than other mammals (19). Amyloidosis has been induced in them experimentally by the injection of dead or living bacteria (2,3,5),

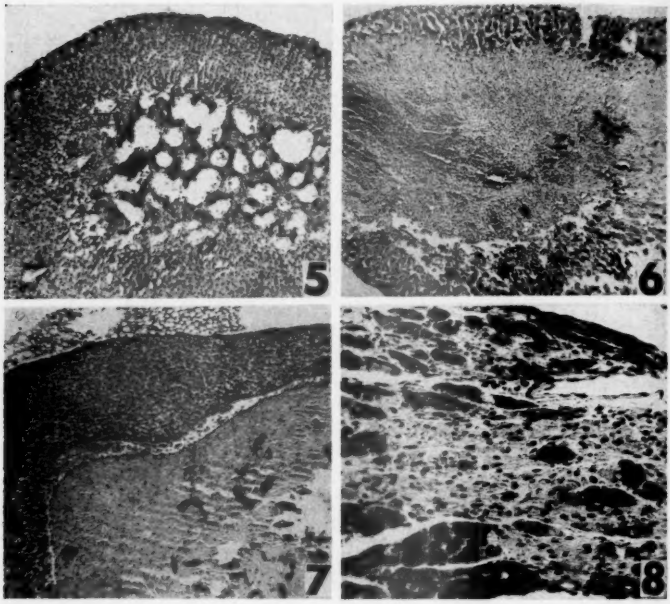

FIG. 5. Adrenal from a mouse which died on the second day of stress exposure. Note that the medulla is occupied by numerous spaces, some confluent. These contain erythrocytes and give to the medulla a "Swiss cheese" appearance. H and E x83.

FIG. 6. Adrenal from a mouse which died after a week of stress exposure. The medulla is occupied by an extensive hemorrhage. Whether this represents a later stage or a more intense development of the preceding change, or is specifically distinct, is uncertain. H and E x83.

FIG. 7. Adrenal from a mouse stressed for 71 days. Medulla is replaced by a granular, rather homogeneous material, walled off from the cortex. H and E x83.

FIG. 8. Heart section from a stressed mouse. Degenerating muscle fibres can be seen, and the intervening spaces are occupied by amyloid deposits and connective tissue fibres. Gomori trichrome x320.

the administration of casein (13), gelatin or egg protein (14), colloidal sulfur (14), phenolic and saline solutions (15), tar painting (22), tumor grafting (16), cysticercus infestation (1) and parabiosis (6). It is difficult to conceptualize a mechanism of action common to such basically dissimilar agencies as these, even granted a susceptible mammalian species. The demonstrated efficacy of exposure to electrical shocks suggests that the common factor might be the induction of a state of stress; particularly

since it has been established that ACTH and cortisone, hormones evoked by stress, are known to aggravate experimental amyloidosis (18). There may, of course, be other physiologic disturbances provoked by stress, but not hormonally mediated, which favor amyloid formation.

Half of the exposed animals escaped amyloidosis. The reason for this is not entirely clear, however it should be emphasized that it is probably the state of stress engendered by the electric shocks rather than the stimuli themselves which is all important. Some mice react much more violently to shocks than others, which appear almost indifferent to them. In retrospect it would have been instructive to ascertain whether reactivity of the mice could have been correlated with the incidence and severity of lesions, but the necessity was not foreseen.

The nature of the influence upon the adrenal of stressed mice which, in some of them, causes the medulla to react with hyperemia, hemorrhage and/or to be replaced by a granular material is uncertain. It would seem probable that the adrenal medulla is intensely stimulated as a result of exposure to chronic stress of the type used, but whether this alone is capable of leading to destruction of the medulla is problematic. Further studies are contemplated in an effort to elucidate this matter.

Summary

Old mice of strain 129 were exposed to periodic electric shocks for a period of 71 days. Males succumbed to this within a few days, whereas females proved able to tolerate the treatment much better.

Half of the animals which survived this treatment for a week or more showed amyloid deposits in the spleen, adrenals, and kidneys, absent from the organs of controls. It is suggested that the factor common to the spectrum of unrelated interventions capable of inducing amyloidosis in the mouse may be stress. An additional finding in many of the exposed animals was hyperemia or hemorrhage of the adrenal medulla, which in extreme cases led to its destruction.

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[b] Since this is given under the heading "organ weights" it seems improper to go into details of pathologic changes, which is done later. However, since pathologic changes affect the weight, some mention of their occurrence should be made.

SOME PROBLEMS IN THE PSYCHOLOGICAL DIAGNOSIS OF BRAIN DAMAGE: AN OVERVIEW[a]

MAURICE KORMAN[b]

It has been said of psychologists in the business of making assessments and predictions that they are often faced with the choice between being vague and being wrong. Which of these two unpleasant alternatives is realized in any given situation is to a large extent a function of the kind of criterion against which we measure our accuracy. When we say of a certain patient that "deep down inside" he is really a dependent, masochistic individual, how can this be proven or disproven in a fairly direct, inexpensive and acceptably valid way? In the psychological diagnosis of brain damage the situation is much improved. In most cases there is available, at least in principle, the unequivocal criteria furnished by exploratory surgery, biopsy or autopsy. Short of that, the thorough neurological examination provides a criterion considerably better than those psychologists usually have to contend with. The tangential question of how good is the neurological examination itself when measured against a higher order criterion such as autopsy data is an interesting one but beyond the scope of this discussion. There is at least some evidence (Fisher and Gonda, 1955) to indicate that the validity of *specific* neurological tools such as pneumoencephalogram, skull X-rays, lumbar puncture, etc. leaves something to be desired. Nevertheless, it can be generally accepted that the overall criteria furnished by the neurologist are reasonably solid, especially when based on examination of the diseased brain itself—a prerequisite of any major research effort.

How then have psychologists fared in this situation where the criteria are not elusive and vagueness no refuge? By absolute standards, not too well. I am thinking, of course, of the controlled studies in literature, discounting for the moment those instances,

[a] Read at the annual meeting of the Texas Psychological Association, 1959, and at the Tuesday Morning Conference of the Department of Psychiatry, University of Texas Southwestern Medical School.

[b] From the Division of Psychology, Department of Psychiatry, University of Texas Southwestern Medical School.

which we well remember because they were so well reinforced, in which by some uncanny intuition we spotted a frontal pole tumor that everybody else had overlooked. We all have such anecdotes, and sometimes it was really so. The truth of the matter, however, is that the history of the psychological diagnosis of brain damage has been characterized by a repetitive pattern —you might almost call it perseverative. A worker publishes an optimistic report of the diagnostic value of a certain set of signs and his data look good. Everybody is happy but the honeymoon, unfortunately, lasts only about a year, which is approximately the duration of the publication lag in most journals today. At that time a second researcher arrives breathlessly upon the scene, cross-validation in hand, with the news that it doesn't work for him or at least not significantly so. Other studies are usually published soon after to administer the *coup de grace*. Yates' review of the literature (1954) abounds in examples of this pattern; almost no well known test is spared. The last five years have seen some improvement but no basic change. What is the effect of this discouraging mass of data on the practicing psychologist? Generally, almost nil. This may be thought to be a discouraging state of affairs by some. However, when you remember the almost neurotic need to brood over the efficiency of our own procedures that characterizes much of the psychological literature, some cynical persons might take this as an indication that our collective ego-strength is quickly catching up to that of older, more established professions. Or it may be, and I offer this suggestion more seriously, that the clinical research on the problem of diagnosing brain damage has been marred by some fundamental errors in approach with the result that we have intuitively, and perhaps not too unwisely, clung to global evaluations which sometimes unaccountably seem to work.

The remainder of this paper is devoted to a cursory examination of some of the problems in this area which appear to be susceptible of solution and ending on something of an optimistic note.

Three major components in the psychological diagnosis of brain damage can be distinguished. The first of these is the *test*. If we define tests broadly enough as to include any careful observation of performance under standard conditions we can assume under this rubric not only the commonly used brain

damage tests but also any other appropriate procedures whether borrowed from neurology, psychophysics or any other discipline with some interest in behavior. The second component concerns the *criterion* about which the test is supposed to permit inferences. Usually this has been phrased as a dichotomy "Yes, there is brain damage" *vs.* "No, there is no brain damage." Obviously, many other inferences are logically possible, such as type of disease process, severity, localization, and so on. The third component involves the *population* that we are dealing with. Obviously any diagnostic activity implies a population to diagnose, some of whom have the diagnosis X, while the others have non-X. This last element involves important problems which have only recently been considered seriously.

I would like to ruminate first about the problem of the criterion. Many studies merely label their experimental group as the "brain damaged group," thus lumping together a number of patients quite heterogeneous in many ways. This, it seems to me, has been a major block to any serious advance in this field. An interesting analogy can be made here between this situation and those tests of neuroticism such as the Woodworth, the House, and the Bernreuter inventories which were popular some 20 and 30 years ago. These tests made the analogous mistake of lumping all neurotics together. They have since faded away but they were doomed from the very first if only because of this homogenizing of all neurotics. It stands to reason that if you sum up the anxiety level of two patients, one an anxiety reaction and the other a conversion reaction, you will end up with an average anxiety level that is probably close to the normal. I have the feeling that some studies which reported that a certain test has no differentiating power in this area, failed to find it because their criterion group included all kinds of brain damaged patients which when averaged were not very different from the control group. A study by Weinstein and Teuber (1957) presents a dramatic example of what can happen when the brain damaged group is characterized a little more carefully. They studied some 60 men who had well localized penetration wounds and on whom Army General Classification Test scores antedating their injury were available. When they compared the difference between the pre- and post-injury testing with the parallel scores of a control group with peripheral nerve injuries, it was found that

the differences between the two groups were not very great. Some two-thirds of the brain injured group improved their scores, many as much as the controls. If they had stopped there, they would have had to report essentially negative results but when they broke down the brain injury group according to wound of entrance by lobe and by hemisphere it turned out that a loss in score upon retesting with this test was related exclusively to damage in the left temporal and the left parieto-temporal areas. And this is still the case even when all cases with a history of aphasic disorder are omitted.

The question now arises, how shall the criterion be broken down? There are many ways of parcelling out the cortex, but they are obviously not all functionally meaningful. The cortex may be subdivided by major lobes, cytoarchitecture, thalamic projection, evoked potential or strychnine neuronography. Cerebral disease or damage can be broken down in many ways: severe *vs.* mild, diffuse *vs.* focal; right hemisphere *vs.* left hemisphere; static *vs.* progressive; or else it can be looked at in terms of the type of pathological process involved: cerebral vascular disease *vs.* tumor *vs.* inflammatory or infectious disease *vs.* demyelinating disease *vs.* trauma, and the subdivisions within each one of these.

My feeling is that at this time we don't really know which of these to choose. Certainly we can avail ourselves of a few guiding principles. To begin with, some of these dimensions are much more likely to be significantly related to our test data than others. I doubt very much if anyone will find a significant relationship between the cytoarchitecture of the cerebrum and Wechsler subtest patterns. We must, however, remember that it is not logically impossible, merely empirically improbable. A second guide could be, what does the neurologist want to know from us? It is likely that a test which discriminates operable gliomas from demyelinating disease would please the neurologist more than a test which permitted, let us say, finer localization on the dimension of caudality. We must be careful, however, that this type of decision oriented investigation does not thrive to the exclusion of careful basic research where the potential payoff may be greater. In any case, the problem of how to break down the criterion remains essentially an empirical one in which all likely candidates should have their day. No substantial re-

search in this area should omit any of them. At the present stage of our knowledge it is better to have a smaller sample of patients on whom we have much reliable and diversified criterion information than it is to have large samples of heterogeneous patients all simply characterized as suffering from "brain damage."

The second critical area is that of the test performance that the psychologist elicits from the brain damaged patient. In practice a surprisingly narrow range of measures is used. Global tests strongly influenced by intellectual factors or emotional changes tend to be favored even though intellectual or emotional test behavior sensitive to slight losses in brain function is likely to be equally susceptible to disturbances having a functional etiology. Although few tests are entirely neutral with reference to these dimensions we should enlarge our repertoire to include measures which are less global and which reflect functioning in more specific modalities. Some workers, notably Teuber and his associates (Teuber, *et. al.*, 1950; Weinstein *et. al.*, 1956; Cohen, 1959; Teuber, 1959) have moved in this direction. More use should be made of multiple dimension batteries including, let us say, adaptation to body tilt, perception of reversible figures, auditory localization, and so on. Although we may have some good hunches as to what may or may not work, and here our knowledge of brain functioning and our past failures can yield some worthwhile hints, there is from an empirical point of view, no end to the type of behavior that may be relevant. In addition to this enriching of the test repertoire, which seems to be going on to some extent, there is need to increase the sophistication with which this larger body of tests is handled. It may be that the most advantageous way of considering the data furnished by a battery of tests is not by looking at them singly in terms of the best cut-off score for each, but to evaluate them in combination as a pattern. There is a hint of the power of this type of approach in a recent publication from Teuber's laboratory (1959, p. 187). Two tests were used, the first involving visual judgment with abnormal posture, and the second the setting of a black thread against an obliquely striped background. When the results of these two tasks are evaluated together, they seem to discriminate a frontal from a parietal group more accurately than when each is considered separately. The recent report of Reitan and Tarshes (1959) of what seems to be an

interaction effect between right *vs.* left cerebral lesions and performance on part A *vs.* B of the Trail Making test seems to likewise emphasize the importance of configural patterns.

Finally, there is no good reason why the elements in our configural pattern should be exclusively test derived. It is historically true that the psychologist's original role was that of a psychometrician. This should not, however, prevent us from utilizing other data in our configurations. Age and sex for instance are important considerations as might be some obvious mental status items. A look at what a neurologist does in his diagnostic activities may help us here. Suppose we give him a symptom or a sign such as vertigo of central etiology. If the patient is 30 years old, the symptom is more likely to point to multiple sclerosis than to be vascular in origin, with the odds reversed if the patient is 60. Offhand, there could seem to be no reason why nature should have exempted psychological tests (which can be thought of as signs or symptoms also) from this type of interactive effect. Statistically, it's obvious that if it is overlooked, the error variance increases. Using such an approach, we might consider a certain diagnostic inference likely in a specific case where the patient (1) had done poorly on test A, D, and E; (2) had done fairly well on test B and C; (3) was between 20 and 30 years of age; (4) showed a clear sensorium and little anxiety—but only if *all* of these conditions held.

Having discussed briefly the test and criterion, I would like now to consider the third component in this outline: the population, or rather the different types of populations, tested. The fact that the validity of a test pattern or cutting score depends partly upon the distribution of the criterion variable or base rates in the clinical population had been almost completely ignored in contemporary research until the now classical paper of Meehl and Rosen (1955). This simply means that a test which is efficient in picking out brain damage when you are working with a neurologist 33% of whose patients, let us say, have cortical brain damage may perform quite differently when you shift to a psychiatric setting where only 7% of the total population turn out to have organic involvement. The importance of this principle, which cannot be questioned, is well illustrated in a careful study by Gilberstadt, Shein and Rosen (1958) using the Archimedes spiral after-effect and paying very close attention

to the characteristics of the various populations on which its efficiency was tested. The consistency with which this fact of life has been overlooked is truly surprising. Even such a careful and expert researcher as Reitan has carried on a research program involving the administration of a standard battery of psychological tests to about 2000 patients over a period of eight years without taking heed of the base rate problem in his reports of proportions of hits and misses (1959, and references therein). It is interesting to note here that not only does the efficiency of cutting scores vary for different populations with different base rates, but it varies also for different decisions within the same population. When using a quick initial screening test, for instance, where we are interested in catching all the "true positives" regardless of how many "false positives" we included, we would have to set our cutting score correspondingly. This leads into a successive hurdles approach, especially applicable where the more valid second instrument is too costly or time consuming while the first screening instrument is easily administered in a routine fashion.

If we take the base rates problem seriously, as we must if our contribution in this area is ever to be substantial, we seem to be faced with a terribly impractical situation. If the intrinsic validity of our tests is closely related to the base rates of the population that we are dealing with, then every psychologist would seem to have to recompute the validity coefficients for his particular type of population. It is possible that this difficulty can be overcome by referring back to a suggestion made by Meehl (1959, p. 121) in a different context. If our composite batteries also include the most predictive kind of life history data available, which might involve such variables as age, education, referral source, and so on, then with some of the population characteristics thus built into our test battery, its generalizability across populations is increased and its applicability in any one situation more readily determined.

In conclusion, I would like to suggest that our potential effectiveness in contributing to the diagnosis and localization of brain damage is great. A break-through can hardly fail if substantial research efforts take into account the following variables: (1) As fine a breakdown on the criterion side as possible, not only in terms of localization but also with reference to various disease

oriented variables; (2) A wide range of the best of global and single modality tests, significant life history data and important mental status information; (3) A large experimental group of considerable heterogeneity together with a series of control groups representing the kind of populations that we are often asked to distinguish the brain damaged patient from; (4) A massive statistical attack on the problems of the patterning or configuration of the tests within our battery and (5) Careful attention of the efficacy of our battery with reference to various populations with different base rates.

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PES CAVUS AND HEREDITARY ATAXIA[a]

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Friedreich (1863) was the first person to distinguish hereditary ataxia from tabes dorsalis but it was not generally accepted as a separate entity until confirmation by Brousse (1882) and by Charcot (1884). In the years that have followed, neurologists have described many distinct hereditary ataxias each with a definite set of clinical signs and symptoms, and names have been applied to many. Thus the type described by Friedreich, which was predominately spinal, with early onset, became known as Friedreich's ataxia.

Through the years some neurologists have advocated a more unified concept of the hereditary ataxias (Eckel and Winkelman '25, Spillane '40). This concept groups various hereditary ataxias together as a unit with clinical variations and it has gained increasing acceptance in recent years (Roth '48, Schut '50, '54, Greenfield '54, Lynch *et al.* '59). It is interesting to note that some of the literature before the turn of the century parallels quite closely some current ideas about the clinical and pathological aspects of this disease as well as its inheritance, though, of course, technical terms of modern genetics had not yet been coined (Whyte, 1898).

It is understandable why distinct types of hereditary ataxia have been described and thought of as separate genetic entities since in a given family the course of the disease often follows a very similar pattern both as to time of onset and clinical course. This, coupled with the discoveries of the concurrently developing discipline of genetics, made it very tempting to assign a different gene to each of the different sets of signs and symptoms, and indeed, some people still hold to this concept (Isaacs, '58). It has been noted by a number of investigators, however, that when large kindred groups are examined many "mixed forms" of the ataxias appear (Schut '50, Woodworth *et al.* '59). Harmony is brought into the occurrence of the condition and into genetic theory by considering hereditary ataxia to be the result of a domi-

nant autosomal gene the expression of which is influenced by unidentified genes (genetic milieu) and perhaps unknown factors in the environment. This can account for both similarities and differences of clinical signs and symptoms within families. Some neuropathologists have also been impressed by the range of distribution of lesions in the hereditary ataxias and think of them as the result of the same basic pathological process (personal communication K. M. Earle). A concise statement of this concept in regard to degenerative neurological diseases has recently been made by Engel, Durland, and Klatzo ('59).

Pes cavus is a foot deformity often found concurrent with hereditary ataxia as well as with other neurological conditions. It is significant that pes cavus is found in all clinical variants of hereditary ataxia. Indeed, it seems to be a link that joins such conditions as Friedreich's ataxia, Charcot-Marie-Tooth disease, so called Roussy-Lévy Syndrome, Marie's cerebellar ataxia, olivopontocerebellar atrophy and hereditary spastic paraplegia (neuromuscular disorders other than Charcot-Marie-Tooth disease where there is no ataxic component). The present study of pes cavus is oriented toward obtaining a better understanding of these variants of hereditary ataxia.

Design of Study

Families in which hereditary ataxia occurred were located by survey letters to the internists, general practitioners and hospitals of the state; by personal letters to the neurologists and neurosurgeons of the state; by a search of hospital records of the Medical Branch Hospitals; and through referrals to the Medical Branch Hospitals and physicians on the staff. Once the diagnosis was made or confirmed in the proband, arrangements were made to see other members of the family. Every family member available was examined if possible and, in general, the families were cooperative. Most kindred were examined in or near their home community in facilities provided by family physicians, county hospitals, state health units, public schools or private homes. Each person was given a neurological and physical examination and additional data were collected. An opinion was recorded by both the neurologist and the physician conducting the physical examination as to the presence or absence of pes cavus and a neurological evaluation was obtained on all kindred in order that each person could be classified as to whether he was or was not affected.

Defining pes cavus is arbitrary. To some a high longitudinal arch is synonymous with pes cavus. Others think that to be classified as such a foot must exhibit in addition a "claw foot" in which the interphalangeal joints are flexed and the metatarsophalangeal joints extended. Schut ('50) sums up

the statements in the literature with "deformities of the feet (in the hereditary ataxias) were variously described as pes cavus, talipes equinus and pes planus." We have observed these and other deformities in ataxic families such as short broad feet, some of which exhibit a stubby or stunted appearance. No way has been found to determine accurately the presence or absence of this condition in mild forms which, undoubtedly, in some cases will become quite obviously affected with the passage of time. Most of the cases of pes cavus recorded in this study were of the extreme type often referred to in the literature as the "Friedreich foot" and the remainder had a high longitudinal arch. The presence or absence of pes cavus was recorded in all persons, both those with hereditary ataxia and those unaffected. Figure 1 shows feet of the type we classify as pes cavus and Figures 2 and 3 are X-rays of such feet in a young adolescent boy and a 62-year-old woman respectively. These illustrations are selected as being typical of our series.

Results

The incidence of pes cavus in persons affected with hereditary ataxia is shown in Table 1. The arrangement is such that the distribution of the foot deformity can be seen in relation to sex, relative age of onset, and clinical signs. Total affected persons number 102 of which 55 were females and 47 were males. Pes cavus occurred in 83 of these (81%). No significant difference occurred

FIG. 1. Photograph of feet of 57 year old woman who has had foot deformity since childhood.

TABLE 1

Incidence of pes cavus in persons affected with hereditary ataxia

		Not having pes cavus Number	Type of sign SC	CS	NM*	Having pes cavus Number	Type of sign SC	CS	NM
under 15 years of age	♂♂	4	3	.	2	15	11	4	5
	♀♀	0	. .	.	.	17	16	0	7
	Total	4	3	.	2	32	27	4	12
over 15 years of age	♂♂	8	8	3	2	20	17	4	16
	♀♀	7	7	2	3	31	29	7	21
	Total	15	15	5	5	51	46	11	37
Grand total		19	18	5	7	83	73	15	49

TOTALS

	By sex	By relative age
Males	35 of 47 or 75%	Under 15 years 32 of 36 or 88%
Females	48 of 55 or 87%	Over 15 years 51 of 66 or 77%
Total	83 of 102 or 81%	

* SC refers to spinocerebellar, CS to corticospinal, and NM to neuromuscular.

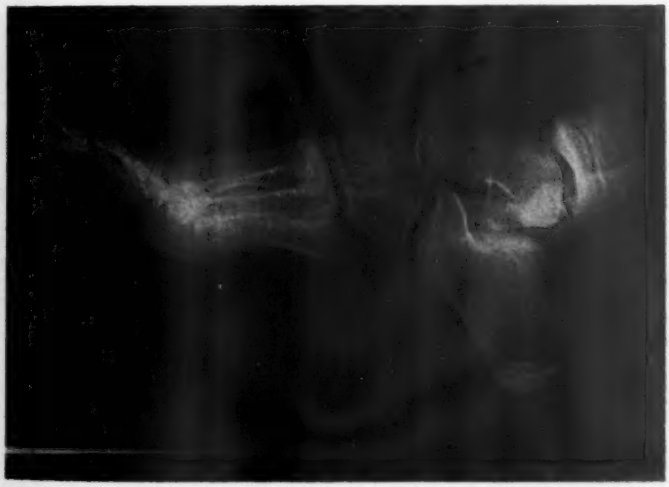

FIG. 2. Photograph of X-ray of foot of 12 year old boy with typical pes cavus.

FIG. 3. Photograph of X-ray of foot of 62 year old woman who has had pes cavus since childhood.

in the incidence of pes cavus in those under fifteen years of age (88%), and in older persons (80%). Likewise, there was no significant sex difference as it occurred in 87 per cent of the affected females and 75 per cent of the affected males, giving an overall occurrence of 81 per cent.

Each affected person was tabulated in one or more of the clinical categories indicated, depending on the clinical signs present. Since many presented clinical manifestations embracing two of the categories and some all three, the total tabulated signs are far in excess of the number of individuals. In all groups regardless of age grouping or sex the spinocerebellar signs are the most frequent, followed in order by the neuromuscular and corticospinal. There is no significant difference between the groups, insofar as pes cavus itself is concerned, though considerably more neuromuscular involvement was found in the group over fifteen years of age.

Table 2 gives the incidence of pes cavus in unaffected relatives of persons afflicted with hereditary ataxia. There are a total of 208 of whom 103 are males and 105 females. Of these, 41 have pes cavus which is 19 per cent of the total. The incidence was

TABLE 2

Incidence of pes cavus in the unaffected kindred of persons with hereditary ataxia

		Having no pes cavus Number	Neurological evaluation Av.	Deviation	Having pes cavus Number	Neurological evaluation Av.	Deviation
under 15 years of age	♂ ♂	41	27	14	7	4	3
	♀ ♀	26	18	8	9	4	5
	Total	67	45	22	16	8	8
over 15 years of age	♂ ♂	42	20	22	13	3	10
	♀ ♀	58	31	27	12	4	8
	Total	100	51	49	25	7	18
Grand total		167	96	71 (81%)	41* (43%)	15 (19%)	26 (63%)

* Of the 41 having pes cavus, males number 20 while females number 21. Sixteen of the individuals under fifteen years of age had pes cavus, or 19%.

essentially the same for those over and those under fifteen years of age and in either sex. The neurological evaluation shows that 43 per cent of persons with no pes cavus had at least one sign outside the normal range. This may be an increased or decreased response of deep tendon reflexes, decreased vibratory or other sense, muscle weakness, incoordination, or the like. In comparison those persons having pes cavus show 63 per cent with at least one neurological sign outside the normal range. In some individuals these were rather great such as a total absence of the knee jerk or of the Achilles reflex.

Discussion

Over a score of causes are given for the occurrence of pes cavus but these can be grouped into six rather large classes: 1) upper motor neurone lesion, 2) lower motor neurone lesion, 3) myopathy, 4) vascular ischemia, 5) congenital defects, and 6) postural adaptations. In a majority of cases of pes cavus, however, the causes are unknown and they are classed as idiopathic. In our experience, some of these have been found to occur in "abortive" forms of hereditary ataxia. In hereditary ataxia the first of the

above causes is primarily responsible. The fact that all affected persons do not develop the foot abnormality may be explained on the basis of chance as to which muscles of the extremities are affected most severely. If the intrinsic muscles of the foot are the more severely weakened, a pes cavus develops, whereas a pes planus may result if muscles of the anterior and lateral aspect of the leg are more severely affected (Isaacs ,'58). In some of our cases of pes cavus, there has been severe atrophy of the intrinsic muscles of the foot, and the "clawing" of the toes is the typical position assumed when loss or weakening of the lumbricales and interossei occur. That a weakening of muscles is not necessary to produce this foot deformity is shown by the fact that if a normal foot is strongly plantar flexed and the toes extended, it assumes the shape of a typical pes cavus. Hyptertonicity of the muscles in disease can also produce this condition. This suggests that the key to its occurrence is in the muscle imbalance of the foot. This may occur with or without hypertrophy, weakness or atrophy. Functional testing of muscles of the leg and foot in individuals in this study revealed that weakness and atrophy occur in some people without pes cavus both in those affected and those not affected with hereditary ataxia. Some persons without pes cavus have stated that they have been unable to walk on their toes or heels since early childhood. A plasticity of the foot is also seen in children with pes cavus where, by manipulation, the relaxed foot can be placed in normal alignment where it remains passively for a few seconds before slowly slipping back into the abnormal position. With long-standing muscle imbalance the calcaneal tendon shortens as does the plantar fascia and other connective tissue resulting. with age, in a more rigid foot. This leads to bunions and calluses or, in extreme cases, even to dorsal dislocation at the metatarsophalangeal joints. This, no doubt, is accentuated by progression of the disease making the muscle imbalance more severe.

Pes cavus in the spinal form of hereditary ataxia is stated by some to be congenital while others find it to be acquired (Brain '51). We have found that both statements are true but most pes cavus is acquired after birth. Tables 1 and 2, show that the pes cavus is well established before fifteen years of age in all the clinical variations presented. Some persons with pes cavus do not

develop ataxia until much later. In fact, we believe some never develop ataxia, even though they live to old age.

The high incidence (19%) of pes cavus in relatives of persons affected with hereditary ataxia (who, themselves, are classed as unaffected) is significant. No statement could be found in the literature giving the incidence of pes cavus in the general population, but on the basis of personal observations in the gross anatomy laboratory for a number of years, this incidence of pes cavus is far in excess of that usually encountered.

A separately inherited genotype for high arch is not a satisfactory explanation for this high incidence since this type of "high arch" is asymptomatic, whereas pes cavus is more extreme in appearance and produces "foot trouble" in those affected. In addition, many of those with pes cavus in ataxic kindred have other neurological signs not seen in persons with inherited "high arches." Th's sometimes consists of absent patellar and calcaneal tendon reflexes even with reinforcement. Pes cavus with so great a neurological deficit undoubtedly indicates a person who has a basic genotype for hereditary ataxia without any other known neurological sign or symptoms of disease. The term "forme fruste" has been used in the medical literature to signify such a condition. Such minimally affected parents usually have some of their children more severely affected than themselves.

There are a few parents in the direct genetic line who have a child affected with the full manifestations of hereditary ataxia who themselves have only pes cavus without other neurological manifestation. In only two instances in our study did we find both parents without neurological stigmata who had produced an affected child. In each of these cases one of the parents was in the direct genetic line and we believe this parent must have the gene for hereditary ataxia but the expression did not reach a clinical level. The above statement is based only on our data where there are family histories of the disease since so called sporadic cases are not included in this study. Also it should be mentioned in this connection that there are a number of other musculo-skeletal deviants and stigmata, some of which are of certain value and others of questionable value in determining whether or not a person is affected with hereditary ataxia. Some of these stigmata of deviation well known to neurologists are kyphosis, scoliosis,

hemiatrophy, asymmetry of cranio-facial bones, asymmetry of ears, "fawn" ears, unusual hair lines, dimpling and hair tufting over the sacrum, webbing of fingers and toes, high arched palate, prognathism, pigmentation, myopathy, dystrophy, and limitation of conjugate ocular movements. These were of no value in identifying "carriers" of this gene in the two "normal" parents mentioned above. It should be mentioned that such deviations would be expected more frequently in children resulting from consanguinous marriages than in the general population and that such marriages occur more frequently in families afflicted with hereditary ataxia (Bell and Carmichael '38).

The literature reports a higher incidence of pes cavus in spinal forms of the ataxia than in cerebellar forms. A recent survey by Brown ('59) of 103 patients admitted in a hospital in 1957 suffering from degenerative cerebellar ataxia reveals only 7 with pes cavus. This large discrepancy in the incidence of pes cavus in this study and ours must be due to the nature of the two populations; one being made up of individuals from the general population and the other composed of relatives from less than a dozen families in which there was a history of hereditary ataxia.

Conclusions

The data collected by examining all relatives of persons affected with hereditary ataxia indicate that pes cavus occurs with similar frequency in hereditary ataxia whether the clinical signs and symptoms are predominately spinocerebellar, corticospinal or neuromuscular. The foot deformity has about the same frequency in those younger and in those older than fifteen years of age, indicating that most persons inheriting genes which produce this condition will develop the foot deformity by fifteen years of age, if they develop at all. Hereditary ataxia is apparently inherited as an autosomal dominant character with a wide range of expression and with almost complete penetrance. The range of expression is from those individuals who are minimally affected with pes cavus or some other minimal neurological deficit with or without pes cavus, to those with extreme debility sufficient to make them bedfast. The disease may result in death at an early age or be compatible with a full active life of normal length.

New criteria may make it possible to identify all persons who have the gene for hereditary ataxia. Some preliminary work on

nerve conduction by one of us (W.L.H.) has shown a slowed velocity in some affected persons. Paper chromatography of urine from several families with hereditary ataxia has shown an unidentified spot which has not been seen in other families. Identification of a metabolic defect would be of value not only in diagnosis of subclinical cases but in suggesting new approaches to treatment.

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EXTRACTS OF PLANTS AND CANCER CHEMOTHERAPY

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A program for screening plants for anticancer activity has continued. Most of the work in this report deals with tumors transplanted in mice. The egg-tumor work for a great number of these plants[b] has been reported previously (McKenna *et al.*, 1959). Plant collection data and some chemical information is available. (Accession Nos. 1001–2000, Wall *et al.*, 1955; Nos. 3001–4000, Wall *et al.*, 1957; Nos. 4001–5000, Wall, *et al.*, 1960; Nos. over 6001 unpublished data).[c] A total of 498 plants were examined.

Experimental Procedure

Extracts used in this work were prepared as shown by Wall *et al.*, 1954. The extracts were tested primarily against tumors transplanted in mice although some were used against egg cultivated tumors. The methods used have been described (Taylor 1953a, 1953b; Taylor *et al.*, 1956).

For tests against tumors cultivated in mice, a dba mouse mammary carcinoma was used. This tumor is known under the designation of RC mammary adenocarcinoma and has been used in screening programs in other laboratories (Tarnowski and Stock, 1955). Mice with transplants of this tumor were given one injection of the test material at or near the tolerated dosage. The effect of the plant extract was evaluated on the basis of the average weight of the tumors from the treated as compared with the average weight of tumors in the control groups for a 10-day test period.

Yolk sacs of embryonated eggs were inoculated through the air sac on the 4th day of incubation with a saline suspension of dispersed tumor tissue. A C_3H mouse mammary carcinoma, especially adapted to growth by this type of cultivation, was used. The test extract was injected over the area vasculosa of the tumor bearing eggs on the 12th day of incubation, or 8 days after the eggs were inoculated with the mouse tumor. The effect of the plant fraction was based on weight differences in the tumors and embryos of the experimental eggs as compared with the controls which developed after treatment with the extracts.

Results

Results are summarized in Tables I through III. Table I reveals the plant extracts that inhibited the growth of tumor transplants

in mice. RC mammary adenocarcinoma implanted in mice is much more difficult to inhibit than the egg cultivated tumor. Only 26 extracts reduced the tumor growth to less than 60% of that of the untreated controls. Animal weight was not seriously affected by the plant extracts. There were 7 plants in the family *Compositae* which inhibited the growth of the mouse cultivated tumors.

A total of 98 plants were tested against egg cultivated tumors. Table II lists 11 plant species which inhibited egg cultivated plant tumor 50% or more. Embryos did not suffer any visible damage during the 24 hour test period. In several instances, embryos of the treated eggs were much larger than the controls. An increase in embryo size is usually associated with a decrease in tumor size. Four species of the family *Betulacea* inhibited the growth of egg cultivated tumors.

Two plants inhibited tumor growth in eggs (McKenna *et al.*, 1959) and mice. They are *Conocarpas erecta* (2128) and *Callitris quadrivalis* (2403). This is a very small percentage of the 498 plants tested in the last two years. Fewer than 5% of the extracts inhibited the growth of tumors cultivated in mice. Approximately 10% of the extracts inhibited the growth of tumors cultivated in fertile eggs.

Discussion

The results extend the frontiers of our search in the plant kingdom for an effective chemotherapy agent just a short distance. A vast amount of the plant kingdom remains untouched, especially of the higher plants. This screening program does indicate a fertile area for further exploration. Promising extracts are being tested against naturally occurring, or spontaneous tumors in mice. This type of testing moves slowly. Results that can be classified as encouraging are rare. However, the program moves ahead in the hope that continued investigation of plant extracts will lead to an effective therapeutic agent.

Summary

Extracts from 498 plants were tested. They all were tested for inhibition of RC mammary adenocarcinoma grown in dba mice. A small number, 98, were tested for inhibition of yolk-sac culti-

vated C_3H mouse mammary carcinoma. Seven plants in the family *Compositae* inhibited tumor cultivated in mice. Four plants from the family *Betulaceae* inhibited the growth of yolk-sac cultivated tumor. Two plants, *Conocarpas erecta* (2128) and *Callitris quadrivalis* (2403), inhibited both types of tumor.

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[c] Contact Dr. Monroe C. Wall, Head, Plant Steroid Investigations, Plant Products Laboratory, same address as [b].

TABLE 1

Plant Extracts Inhibiting the Growth of RC Mammary Carcinoma Transplants in dba Mice. One Subdermal Injection. 10-Day Test Period.

Family	Plant	USDA Accession No.	No. Expts.	No. Mice	Dosage Mg. of Dried Material	Tumor Growth at Autopsy, Control = 100	Body Weight at Autopsy, Control = 100
Anacardiaceae	Rhus typhina	4659	2	18	10.4	46	100
Combretaceae	Conocarpus erecta	2128	2	17	12.5	48	101
Combretaceae	Terminalia bellerica	2072	2	18	6.4	47	99
Compositae	Aster pilosus var. demotus	4480	2	18	10.0	55	93
Compositae	Coreopsis lanceolata	4076	2	18	18.9	45	98
Compositae	Cirsium arvense	4809	2	18	21.3	59	96
Compositae	Echinacea pollida	4636	2	18	20.0	36	98
Compositae	Ratibida pinnata	4616	3	27	23.8	47	104
Compositae	Solidago fistulosa	4464	2	18	10.0	24	95
Compositae	Solidago gigantea	4553	2	18	10.9	52	97
Compositae	Tanacetum vulgare	4816	2	18	20.4	20	100
Ericaceae	Chamaedaphne calyculata	4410	2	18	11.1	49	98
Ericaceae	Menziesia pilosa	4663	2	18	22.7	51	96
Ericaceae	Polycodium floridanum	3798	2	18	7.2	44	97
Myrtaceae	Eugenia jambos	2313	2	18	2.4	47	98
Palmae	Acoelorhaphe arborescens	4710-A	2	18	10.0	35	93
Palmae	Aeria attenuata	4224-C	2	18	20.8	59	110
Pinaceae	Callitris quadrivalis	2403	2	18	5.2	46	98
Rosaceae	Geum aleppicum var. strictum	4815	2	18	20.4	57	99
Rosaceae	Physocarpus opulifolius	4394	2	18	22.2	59	99
Rutaceae	Citrus amblycarpa	2058	2	18	9.6	52	102
Sapindaceae	Sapindus senegalensis	2071	3	27	24.3	58	98
Solanaceae	Cestrum parqui	4379-A	2	18	5.0	27	96
Solanaceae	Cestrum parqui	4379-B	2	18	11.6	45	102
Solanaceae	Dunalia campanulata	3905	2	18	22.2	47	99
Solanaceae	Lycium halimifolium	4097	2	18	4.1	47	100

TABLE 2

Plant Extracts Inhibiting the Growth of C_3H Mammary Carcinoma in Tumor Bearing Eggs. 24-Hour Test Period.

Family	Plant	USDA Accession No.	No. Expts.	No. Eggs	Dosage Mg. of Dried Material	Survival Control = 100	Avg. Growth for Test Period. Control = 100	
							Tumor	Embryo
Acanthaceae	Jacobinia coccinea	2269	5	208	14.28	83	39	151
Anacardiaceae	Pistacia lentiscus	4153	2	68	15.4	88	5	99
Betulaceae	Almus serrulata	3929	2	54	21.6	100	22	119
Betulaceae	Carpinus caroliniana	4325	2	54	22.7	100	–8	105
Betulaceae	Ostrya virginiana	4526	2	53	23.2	100	50	116
Bromeliaceae	Bromelia pinguin	2562	2	53	8.4	100	50	118
Bromeliaceae	Pitcairnia corallina	2318	2	56	13.3	100	30	124
Elaegnaceae	Elaeagnus philippensis	2217	2	48	18.8	84	4	143
Melastomotaceae	Tetrazygia bicolor	2728	3	80	20.8	84	43	110
Meliaceae	Trichilia hirta	2697	3	79	19.6	80	0	139
Moraceae	Ficus aurea	2137	4	176	20.8	83	–9	121

TABLE 3

Plant Extracts with Little or No Effect on RC Mammary Carcinoma (9- to 10-Day Tests) and Egg Cultivated Tumors (24-Hour Test)

Family	Plant	USDA Accession No.
Acanthaceae	Crossandra infundibuliformis	2628
Acanthaceae	Thunbergia grandiflora	2029
Amaryllidaceae	Agave atrovirens	1765
Amaryllidaceae	Agave expansa	1003
Amaryllidaceae	Agave mescal	1269
Amaryllidaceae	Agave brachystachya	1747
Amaryllidaceae	Agave desertii	1464
Amaryllidaceae	Agave desertii	1463
Amaryllidaceae	Agave fourcroydes	1761
Amaryllidaceae	Furcraea cabuya	3437
Amaryllidaceae	Furcraea guatamalensis	3152
Amaryllidaceae	Manfreda brachystachys	3137
Amaryllidaceae	Manfreda maculata	3072
Amaryllidaceae	Manfreda pringlei	3080
Amaryllidaceae	Manfreda sp.	3020
Amaryllidaceae	Manfreda sp.	3051
Anacardiaceae	Anacardium excelsum	2529
Anacardiaceae	Anacardium sp.	2651
Anacardiaceae	Cyrtocarpa procera	3016
Anacardiaceae	Loxopterygium huasango	2701
Anacardiaceae	Metopium toxiferum	2112
Anacardiaceae	Pistachia chinensis	6103-A
Anacardiaceae	Pistachia chinensis	6103-B
Anacardiaceae	Pistachia chinensis	6103-C
Anacardiaceae	Pistachia chinensis	6103-C1
Anacardiaceae	Pistachia vera	6121-A
Anacardiaceae	Pistachia vera	6121-B
Anacardiaceae	Pistacia lentiscus	4153
Anacardiaceae	Pistacia lentiscus	6123-A
Anacardiaceae	Pistacia lentiscus	6123-A1
Anacardiaceae	Pistacia lentiscus	6123-B
Anacardiaceae	Pistacia terebinthus	6127-A
Anacardiaceae	Pistacia terebinthus	6127-A1
Anacardiaceae	Pistacia terebinthus	6127-B
Anacardiaceae	Pistacia terebinthus	6127-B1
Anacardiaceae	Pistacia terebinthus	6127-C
Anacardiaceae	Pistacia chinensis	1705
Anacardiaceae	Pleiogynium solandri	2127
Anacardiaceae	Poupartia axillaris	2702
Anacardiaceae	Spondias purpurea	2829
Anacardiaceae	Rhus aromatica	4051
Anacardiaceae	Rhus copallina	4073
Anacardiaceae	Rhus glabra	4082
Anacardiaceae	Rhus typhina	4659
Anacardiaceae	Rhus ovata	4787
Anacardiaceae	Rhus vernix	4286
Anacardiaceae	Schinopsis lorentzii	3056
Anacardiaceae	Schinus dependens	2840
Anacardiaceae	Schinus Polygamus	2841
Annonaceae	Annona paludosa	2839
Apocynaceae	Allamanda violacea	4712-A
Apocynaceae	Allamanda violacea	4712-B
Apocynaceae	Alstonio scholaris	2319

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Apocynaceae	Amsonia ciliata	4052
Apocynaceae	Beaumontia grandiflora	2467
Apocynaceae	Funtumia elastica	1668
Apocynaceae	Funtumia sp.	2186-A
Apocynaceae	Funtumia sp.	2186-B
Apocynaceae	Funtumia sp.	2216
Apocynaceae	Ochrosia elliptica	2266
Apocynaceae	Rauvolfia tetraphylla	2941
Apocynaceae	Stronphanthus gratus	1671
Apocynaceae	Strophanthus gratus	2187-B
Apocynaceae	Vallaris solanacea	2216
Apocynaceae	Vinca minor	2181
Araceae	Colocasia sp.	2282-A
Araceae	Zantedeschia aetheopica	2660
Asclepiadaceae	Hoya globulosa	2891
Ascledpiadaceae	Periploca sp.	2303
Betulaceae	Betula lenta	4507
Betulaceae	Betula nigra	4083
Betulaceae	Betula populifolia	4845
Betulaceae	Corylus americana	4393
Betulaceae	Ostrya virginiana	4666
Bignoniaceae	Godmania aesculifolia	2844
Bignoniaceae	Kigelia pinnata	2129
Bignoniaceae	Parmentiera cereifera	2857
Bignoniaceae	Parmentiera edulis	2644
Bignoniaceae	Stereospermum euphorioides	2863
Bignoniaceae	Tabebuia chrysea	2950
Bignoniaceae	Tabebuia glomerata	2523
Bignoniaceae	Tabebuia leucoxyla	2721
Bombaceae	Adansonia digitata	2845
Bombaceae	Atkinsia cubensis	2218
Bombaceae	Ceiba acuminata	2944
Bombaceae	Ceiba parvifolia	2943
Bombaceae	Pachira aquatica	2961
Bombaceae	Pachira fastuosa	2521
Boraginaceae	Cordia brachycalyx	2967
Boraginaceae	Cordia leucosebestena	2992
Boraginaceae	Cordia tremula	2955
Bromeliaceae	Tillandsia usneoides	2120
Burseraceae	Bursera graveolens	2126
Canellaceae	Canella winterana	2113
Capparidaceae	Cleome spinosa	4786
Capparidaceae	Polanisia graveolens	4835
Capparidaceae	Ritchiea reflexa	2494
Caprifoliaceae	Lonicera maackii	3937
Caprifoliaceae	Symphoricarpus orbiculatus	4640
Caprifoliaceae	Viburnum acerifolium	4853
Cochlospermaceae	Cochlospermum vitifolium	2214
Combretaceae	Bucida buceras	2534
Combretaceae	Combretum farinosum	2497
Combretaceae	Combretum grandiflorum	2042
Combretaceae	Conocarpas erecta	2128
Combretaceae	Quisqualis indica	2825
Combretaceae	Terminalia arjuna	1612
Combretaceae	Terminalia bellerica	2072
Combretaceae	Terminalia catappa	2849

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Compositae	Acanthospermum australe	4593
Compositae	Achillea millefolium	4043
Compositae	Actinomeris alternifolia	4543
Compositae	Ambrosia artemisiifolia var. paniculata	4317
Compositae	Anaphalis margaritacea	4839
Compositae	Antennaria plantaginifolia	4130
Compositae	Anthemis arvensis	4301
Compositae	Artemisia absinthium	4629
Compositae	Artemisia ludoviciana	4527
Compositae	Artemisia tridentata var. angustifolia	4793
Compositae	Arctium lappa	4279
Compositae	Arctium minus	4539
Compositae	Aster lateriflorus	4307
Compositae	Aster pilosus var. demotus	4480
Compositae	Aster puniceus	4535
Compositae	Baccharis angustifolia	3791
Compositae	Baccharis halimifolia	3726
Compositae	Balduina uniflora	4974
Compositae	Berlandiera pumila	4962
Compositae	Bidens bipinnata	4486
Compositae	Bidens pilosa	4873
Compositae	Bigelowia nudata	4990
Compositae	Boltonia diffusa	4340
Compositae	Borrichia frutescens	3792
Compositae	Cacalia atriplicifolia	4244
Compositae	Cacalia elliottii	4981
Compositae	Cacalia suaveolens	4558
Compositae	Carduus acanthoides	4528
Compositae	Carphephorus tomentosus	4422
Compositae	Centaurea maculosa	4520
Compositae	Centaurea solstitialis	4643
Compositae	Chrysanthemum leucanthemum	0-6088
Compositae	Chrysogonum virginicum	3913
Compositae	Chrysopsis gossypina	4419
Compositae	Chrysopsis graminifolia	4421
Compositae	Chrysopsis microcephala	5000
Compositae	Cirsium altissimum	4538
Compositae	Cirsium vulgare	4483
Compositae	Clibadium divaricatum	3974
Compositae	Coreopsis helianthoides	4982
Compositae	Coreopsis lanceolata	4076
Compositae	Coreopsis major var. stellata	4323
Compositae	Echinacea pallida	4636
Compositae	Echinacea purpurea	4647
Compositae	Eclipta alba	4702
Compositae	Elephantopus carolinianus	4425
Compositae	Elephantopus elatus	4876
Compositae	Erigeron annus	3841
Compositae	Erigeron canadensis	4243
Compositae	Erigeron pusillus	4766
Compositae	Eupatorium album	4960
Compositae	Eupatorium capillifolium	4266
Compositae	Eupatorium compositifolium	4361
Compositae	Eupatorium cordigerum	4332

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Compositae	Eupatorium cuneifolium	4770
Compositae	Gaillardia pulchella	3730
Compositae	Galinsoga parviflora	4282
Compositae	Haplopappus divaricatus	4775
Compositae	Helianthus microcephalus	4331
Compositae	Helianthus mollis	4632
Compositae	Heliopsis helianthoides	4518
Compositae	Helenium godfreyi	4495
Compositae	Helenium tenuifolium	4329
Compositae	Heterotheca latifolia	4565
Compositae	Heterotheca subaxillaris	3649
Compositae	Hieracium venosum	4131
Compositae	Hypochaeris radicata	4392
Compositae	Inula helenium	4665
Compositae	Iva asperifolia	4971
Compositae	Iva frutescens	3727
Compositae	Iva imbricata	4472
Compositae	Liabum pallatangense	4027
Compositae	Liatris chapmanii	4891
Compositae	Liatris graminifolia	4421
Compositae	Liatris laevigata	4890
Compositae	Melanthera hastata	4967
Compositae	Mikania scandens	4590
Compositae	Parthenium hysterophorus	4875
Compositae	Parthenium integrifolium	4170
Compositae	Pluchea camphorata	4948
Compositae	Pluchea foetida	4936
Compositae	Pluchea longifolia	4970
Compositae	Polymnia uvedalia	4218
Compositae	Pterocaulon undulatum	4177
Compositae	Pyrrhopappus carolinianus	3790
Compositae	Ratibida pinnata	4616
Compositae	Rudbeckia laciniata	4516
Compositae	Rudbeckia serotina	4189
Compositae	Senecio aureus	4991
Compositae	Senecio smallii	3690
Compositae	Sericocarpus asterioides	4215
Compositae	Sericocarpus tortifolius	4418
Compositae	Silphium asteriscus	4321
Compositae	Silphium compositum	4257
Compositae	Solidago altissima	4322
Compositae	Solidago boottii var. caroliniana	4764
Compositae	Solidago fistulosa	4464
Compositae	Solidago gigantea	4553
Compositae	Sonchus asper	3709
Compositae	Sonchus oleraceus	4825
Compositae	Spilanthes americana	4029
Compositae	Tanacetum vulgare	4816
Compositae	Tetradymia comosa	4796
Compositae	Tragopogon pratensis	4692
Convolvulaceae	Porana paniculata	2043
Crassulaceae	Echeveria sp.	2160
Dioscoreaceae	Dioscorea composita	3200
Dioscoreaceae	Dioscorea spiculiflora	2932
Ebenaceae	Diospyros kaki	1850

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Ebenaceae	Diospyros kaki	1939
Ebenaceae	Maba inconstans	2956
Ericaceae	Arctostaphylos pungens	3557
Ericaceae	Arctostaphylos pungens	3558
Ericaceae	Chamaedaphne calyculata	4410
Ericaceae	Epigaea repens	4397
Ericaceae	Gaylussacia tomentosa	4067
Ericaceae	Kalmia angustifolia	4302
Ericaceae	Leiophyllum buxifollium	4411
Ericaceae	Leucothoe axillaris	3732
Ericaceae	Lyonia lucida	3592
Ericaceae	Menziesia pilosa	4663
Ericaceae	Pieris japonica	2087-A
Ericaceae	Pieris japonica	2087-B
Ericaceae	Polycodium floridanum	3798
Ericaceae	Pyrola rotundifolia	4658
Ericaceae	Rhododendron canadense	4843
Ericaceae	Rhododendron viscosum	4405
Ericaceae	Vaccinium arboreum	3799
Ericaceae	Vaccinium corymbosum	4840
Ericaceae	Vaccinium macrocarpon	4916
Ericaceae	Zenobia pulverulenta forma nuda	4196
Euphorbiaceae	Alchornia cordifolia	3057
Euphorbiaceae	Euphorbia cotinifolia	2936
Euphorbiaceae	Garcia nutans	1860
Euphorbiaceae	Hoppomane mancinella	2023
Euphorbiaceae	Jatropha curcas	2730
Euphorbiaceae	Joannesia princeps	2633
Euphorbiaceae	Phyllanthus emblica	2423
Euphorbiaceae	Phyllanthus grandifolius	2937
Euphorbiaceae	Putranjiva roxburghii	2555
Euphorbiaceae	Sapium sebiferum	1410
Euphorbiaceae	Savia sessiliflora	1837
Fagaceae	Quercus lobata	2491
Flacourtiaceae	Flacourtia montana	2362
Gramineae	Bromus catharticus	3936
Gramineae	Neyraudia madagascariensis	3655
Gramineae	Themeda gigantea	3653
Gramineae	Zoysia japonica	3651
Guttiferae	Calophyllum inophyllum	2901
Guttiferae	Garcinia binucao	2888
Guttiferae	Garcinia spicata	2823
Hernandiaceae	Hernandia peltata	2935
Juglandaceae	Carya cordiformis	4739
Juglandaceae	Carya tomentosa	4198
Juglandaceae	Juglans nigra	4209
Leguminosae	Abrus precatorius	2116
Leguminosae	Acacia catechu	1669
Leguminosae	Acacia stricta	1178
Leguminosae	Acacia spadicigera	1303
Leguminosae	Acrocarpus fraxinifolius	2847
Leguminosae	Adenanthera microsperma	2954
Leguminosae	Albizzia julibrissin	1258
Leguminosae	Albizzia kalkora	1260
Leguminosae	Andira inermis	2024
Leguminosae	Bauhinia galpinii	1582

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Leguminosae	Bauhinia tomentosa	1613
Leguminosae	Brownea coccinea	2308
Leguminosae	Butea capitata	1418
Leguminosae	Caesalpinia ferrea	2060
Leguminosae	Calliandra haematocephala	1583
Leguminosae	Calliandra surinamensis	2038
Leguminosae	Caragana microphylla	1920
Leguminosae	Cassia alata	1949
Leguminosae	Cladrastis amurensis	1566
Leguminosae	Colvillea racemosa	1943
Leguminosae	Cytisus supinus	1913
Leguminosae	Dalbergia sissoo	2073
Leguminosae	Derris elliptica	2563
Leguminosae	Enterolobium timbouva	1829
Leguminosae	Gleditsia triacanthos	1400
Leguminosae	Gymnocladus chinensis	1681
Leguminosae	Hebestigma cubense	2850
Leguminosae	Lathyrus latifolius	1907
Leguminosae	Leucaena glauca	2198
Leguminosae	Mundulea suberosa	1575
Leguminosae	Olneya tesota	1483
Leguminosae	Peltophorum brasiliense	1834
Leguminosae	Petteria ramentacea	1926
Leguminosae	Piptadenia macrocarpa	1675
Leguminosae	Piscidia mollis	1335
Leguminosae	Pithecellobium brevifolium	1300
Leguminosae	Pithecellobium flexicaule	1619
Leguminosae	Vigna sinensis	1565-A
Liliaceae	Allium haematochiton	2621
Liliaceae	Aloe barbadensis	2309
Liliaceae	Anthericum sp.	3017
Liliaceae	Asparagus dauricus	1916
Liliaceae	Beaucarnea inermis	3197
Liliaceae	Chlorogalum pomeridianum	2283
Liliaceae	Cordyline terminalis	1299
Liliaceae	Eucomis undulata	2653
Liliaceae	Gloriosa rothschildiana	2852
Liliaceae	Gloriosa superba	2853
Liliaceae	Hesperaloe funifera	2332
Liliaceae	Milla biflora	1095
Liliaceae	Nolina matapensis	2384
Liliaceae	Ornithogalum thyrsoides	2654
Liliaceae	Sansevieria zeylanica	2794
Liliaceae	Schoenocaulon officinale	2726
Liliaceae	Smilax herbacea	1593
Liliaceae	Urginea burkei	3401
Liliaceae	Veratrum viride	1042
Lythroceae	Lagerstroemia speciosa	2900
Malpighiaceae	Byrsonima spicata	2800
Malvaceae	Bombycidendron vidalianum	2133
Malvaceae	Hisbiscus cannabinus	1454
Malvaceae	Kosteletzkya virginica	4477
Malvaceae	Kydia calycina	2041
Malvaceae	Malva moschata	4600
Malvaceae	Malva rotundifolia	4805
Malvaceae	Malvaviscus mollis	1783

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Malvaceae	Modiola caroliniana	3780
Malvaceae	Sida sp.	3845
Meliaceae	Cedrela odorata	2819
Meliaceae	Khaya nyasica	2865
Meliaceae	Melia azedarach	2027
Meliaceae	Swietenia candollei	2641
Meliaceae	Swietenia humilis	2642
Meliaceae	Trichilia chirindensis	2699
Meliaceae	Trichilia spondiodes	2953
Moraceae	Broussonetia papyrifera	3036
Moraceae	Ficus altissima	3670-A
Moraceae	Humulus japonicus	4254
Myricaceae	Myrica cerifera	3588
Myricaceae	Myrica rubra	1405
Myrtaceae	Eucalyptus triantha	3095
Myrtaceae	Eugenia jambos	2313
Oleaceae	Forestiera rhamnifolia	1833
Oleaceae	Forsythia sp.	1812
Oleaceae	Fraxinus americana	4203
Oleaceae	Fraxinus caroliniana	4175
Oleaceae	Jasminum humile	4096
Oleaceae	Jasminum pubescens	1977
Oleaceae	Ligustrum lucidum	3832
Oleaceae	Ligustrum sinense	3813
Oleaceae	Ligustrum vulgare	3844
Oleaceae	Noronhia emarginata	1832
Oleaceae	Olea verrucosa	2995
Oleaceae	Osmanthus americanus	3594
Oleaceae	Osmanthus ilicifolius	2082
Oleaceae	Syringa oblata var. affinis	2592
Palmae	Acanthorhiza aculeata	3652
Palmae	Acoeloraphe arborescens	4710-A
Palmae	Acrocomia sclerocarpa	3654
Palmae	Adonidia merrillii	2132
Palmae	Aeria attenuata	4224-A
Palmae	Aeria attenuata	4224-B
Palmae	Aeria attenuata	4224-C
Palmae	Areca catechu	4718-A
Palmae	Areca catechu	4718-B
Palmae	Areca catechu	4718-C
Palmae	Arecastrum romanzoffianum	2138
Palmae	Arikuryroba schizophylla	2200
Palmae	Attalea cohune	2069
Palmae	Bismarckia nobilis	2468
Palmae	Brahea dulcis	2557
Palmae	Butia bonnetii	2052
Palmae	Butia capitata var. odorata	2626
Palmae	Butia nehrlingiana	2146
Palmae	Caryota plumosa	2740
Palmae	Chrysalidocarpus lutescens	2739
Palmae	Chrysalidocarpus madagascariense	1790
Palmae	Coccothrinax argentea	2538
Palmae	Coccothrinax barbadensis	2577
Palmae	Coccothrinax crinita	1298
Palmae	Coccothrinax garberi	2627
Palmae	Cocos australis	3563

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Palmae	Cocos nucifera	2738
Palmae	Copernicia cerifera	2530
Palmae	Dictyosperma grandiformis	1428
Palmae	Dictyosperma rubrum	2645
Palmae	Glaucothea armata	2559
Palmae	Hyophorbe amaricaulis	2131
Palmae	Hyophorbe verschaffeltii	2262
Palmae	Hyphaena turbinata	2263
Palmae	Inodes texana	2742
Palmae	Latania commersonii	2028
Palmae	Latania loddigesii	2469
Palmae	Linoma alba var. aurea	2141
Palmae	Opsiandra maya	2271
Palmae	Orbignya speciosa	2130
Palmae	Paurotis wrightii	2737
Palmae	Pelagodoxa henryana	2741
Palmae	Phoenix canariensis	3560
Palmae	Phoenix canariensis	3562
Palmae	Phoenix farinifera	1861
Palmae	Phoenix pusilla	2536
Palmae	Phoenix roebelenii	2648
Palmae	Phoenix zeylanica	2808
Palmae	Pseudophoenix saonae	2144
Palmae	Pseudophoenix sargentii	2143
Palmae	Pseudophoenix vinifera	3659
Palmae	Ptychosperma macarthuri	2142
Palmae	Sabal longepedunculata	2147
Palmae	Sabal palmetto	1791
Palmae	Seaforthia elegans	2732
Palmae	Seaforthia elegans	2213
Palmae	Serenoa repens	4886
Palmae	Thrinax microcarpa	2810
Palmae	Thrinax morrisii	2745
Palmae	Thrinax wendlandiana	1429
Palmae	Tilmia caryotaefolia	2558
Phytolaccaceae	Limeum aethiopicum	3517
Phytolaccaceae	Steghosperma halimifolium	3276
Pinaceae	Abies pungens	2089
Pinaceae	Callitris quadrivalvis	2403
Pinaceae	Cedrus atlantic	2097
Pinaceae	Chamaecyparis pisifera plumosa	2091
Pinaceae	Cupressus benthami	2078
Pinaceae	Cupressus funebris	2169
Pinaceae	Cupressus sempervirens	2170
Pinaceae	Juniperus barbadensis	2053
Pinaceae	Picea omorika	2088
Pinaceae	Pinus palustris	3591
Pinaceae	Pinus taeda	3678
Pinaceae	Pseudotsuga taxifolia	2183
Rosaceae	Agrimonia gryposepala	4531
Rosaceae	Amelianchier arborea	3836
Rosaceae	Aruncus dioicus	4603
Rosaceae	Chrysobalanus icaco	2359
Rosaceae	Duchesnea indica	3807
Rosaceae	Geum aleppicum var. strictum	4815
Rosaceae	Gillenia stipulata	4626

TABLE 3 *(Continued)*

Family	Plant	USDA Accession No.
Rosaceae	Malus kaido	1628
Rosaceae	Physocarpus opulifolius	4394
Rosaceae	Potentilla canadensis	3812
Rosaceae	Poterium sanguisorba	3118
Rosaceae	Prunus angustifolia	3931
Rosaceae	Rubus argutus	4327
Rosaceae	Rubus cuneifolius	4767
Rosaceae	Rubus phoenicolasius	4667
Rutaceae	Atalantia citroides	4714-A
Rutaceae	Atalantia citroides	4714-B
Rutaceae	Atalantia citroides	4714-C
Rutaceae	Casimiroa edulis	2117
Rutaceae	Citropsis gilletiana	2815
Rutaceae	Citrus amblycarpa	2058
Rutaceae	Pamburus missionis	2813
Rutaceae	Phellodendrom sp.	1363
Rutaceae	Poncirus trifoliata	2814
Rutaceae	Swinglea glutinosa	2466
Sapindaceae	Alectryon subcinereum	2650
Sapindaceae	Dodonaea viscosa	3003
Sapindaceae	Sapindus indica	2858
Sapindaceae	Sapindus senegalensis	2071
Sapindaceae	Sapindus utilis	2480
Sapindaceae	Serjania mexicana	2561
Sapotaceae	Bumelia tenax	3783
Sapotaceae	Chrysophyllum cainito	2500
Sapotaceae	Chrysophyllum oliviforme	2501
Sapotaceae	Madhuca indica	3604
Sapotaceae	Malacantha warneckeana	2698
Solanaceae	Cestrum parqui	4379-A
Solanaceae	Cestrum parqui	4379-B
Solanaceae	Datura stramonium	4485
Solanaceae	Duboisia leichardtii	2905
Solanaceae	Dunalia campanulata	3905
Solanaceae	Lycium halimifolium	4097
Solanaceae	Physalis maritima	4769
Solanaceae	Physalis viscosa	4054
Solanaceae	Solanum abutiloides	1936-C
Solanaceae	Solanum dulcamara	4134
Solanaceae	Solanum nigrum	4738
Solanaceae	Solanum sp.	1989
Solanaceae	Solanum sp.	3548
Solanaceae	Solanum sp.	3618
Solanaceae	Solanum sp.	3939
Symplocaceae	Symplocos tinctoria	4433
Theaceae	Gordonia lasianthus	3590

A NOTE ON THE FINE STRUCTURE OF ENDOTHELIUM IN OLD MICE

RICARDO MORALES, DONALD DUNCAN and DAVID NALL

Although there have been numerous accounts (1,2,3,5,10) of membranous whorls, myelin figures and laminated corpuscles in a variety of cell types, no record has been found of their presence in capillary endothelium. The lack of a previous description and the fact that such objects have not been seen in endothelium of younger animals during numerous studies in this laboratory, suggest that the structures described in this note are associated with the aging process. The younger material referred to consists of endothelium in the dorsal root ganglia, the carotid body, and the hypophysis of mice under six months of age. This possibility is fortified by the presence of typical old age pigment in the capillary endothelium of old mice.

Materials and Methods

Dorsal root ganglia from mice 18 months old were excised and immediately fixed in Dalton's mixture (4) for 1 hour. After fixation the ganglia were rinsed in distilled water and passed through graded alcohols starting with 10% and proceeding by 10% steps to absolute. The material was embedded in a mixture of 85% n-butyl methacrylate and 15% methyl methacrylate, sectioned with a Porter-Blum microtome using glass knives, and studied and photographed in an RCA EMU-3C microscope.

Observations

The objects to be described were first noticed by one of us (R.M.) while photographing old-age pigment in nearby neurons. While they are not seen in every section through a capillary, arteriole or venule they are quite numerous and can be found in any sample of the material containing several small blood vessels. Occasionally, as illustrated in Figure 1, two or more of these bodies occur in the endothelium of a single vessel.

The presumably abnormal bodies may be described as whorls of membranes surrounding a core which differs considerably from one body to another. The most frequent type which is depicted in Figure 2 contains a dense core which is structureless

under the conditions of study. Other whorls surround a bit of cytoplasm loaded with granules of a size and density that permits calling them granules of Palade (7) or ribosomes.

Still others surround one or more mitochondria (Fig. 4). While most of the whorls are small enough to be embedded in endothelium of moderate thickness a few are so large that they obscure the lumen of the vessel (Fig. 6). In this connection it must be kept in mind that excision and subsequent fixation is an inadequate method for determining the size of a lumen or the thickness of the endothelium. Some contraction and collapse of the vessels must be assumed under these conditions.

The dense more or less concentric lines are thought to be single membranes viewed on edge. This opinion is based on numerous photographs exemplified by Figures 3 and 6 showing relatively wide translucent ribbons which are continuous with the characteristic narrow dense lines. The ribbons are interpreted as sections of membrane placed obliquely and perhaps nearly parallel to the plane of section. The ribbon shapes may be due to collapse of on-edge membranes as a result of sublimation of the methacrylate under the electron beam as suggested by Revel, Ito and Fawcett (8). However, it appears reasonable to think this appearance also reflects undulations and changes in plane of the membranes in the living state. The individual membranes display a pronounced tendency to associate in pairs, see Fig. 2. Each pair is separated by a fairly uniform interval of low density and the pairs are separated by wider and more irregular intervals of low density. The significance of the pairing is unknown but it no doubt means that one surface of each membrane is different from the other.

In addition to the membranous whorls, the endothelium in this material contains small amounts of old age pigment which is structurally identical to that seen in the adjacent nerve cells. This material is illustrated in Figure 5.

In other respects the endothelial cytoplasm is essentially as described by Moore and Ruska (6) and many other authors. The micropinocytotic vesicles characteristic of endothelium are present in abundance (Fig. 6). The fact that the interior of most of these vesicles is denser than the cytoplasm may be of some significance but additional study would be required for any statement beyond a passing comment.

Discussion

The significance of concentric paired membranes in cytoplasm is incompletely understood at the present time. According to Fawcett and Ito (5) who reviewed the subject in 1958 they have been seen more often than not in abnormal cells and in normal cells subjected to an adverse environment. Under the name of myelin bodies they are described by Bencosme et al. as an abnormality of glomerular epithelium suffering from acute uranyl nitrate poisoning. On the other hand, they are described by Cotte (3) as normal features of adrenal cortical cells. Consequently, it seems reasonable to think that considerably more information is needed before any definite statements can be made on the significance of myelin figures in the cytoplasm. No doubt they are a normal feature of some cells and definitely abnormal in others. Nevertheless, Fawcett and Ito's experimental production of the whorls by isolating testicular cells and allowing them to stand for up to four hours makes it advisable to suspect adversity of the environment or abnormality of the cell whenever such bodies are seen. This is especially true of the present observations because myelin figures or membranous whorls should by now be a well recognized feature of vascular endothelium if these bodies were at all common in capillaries from young animals. It is the absence of previous descriptions and the association of these structures with typical old age pigment that leads to the suggestion that age is a factor in their production in endothelium. Both features, but especially the pigment, raise questions as to longevity of endothelial cells. Are they in some locations such as the nervous system as old as the neurons they nourish?

The source and mode of development of the whorls seen in endothelium can only be suggested. The evidence of Fawcett and Ito pointing to the endoplasmic reticulum as the source is convincing. This origin is also subscribed to by Bradbury and Meek (2). Inclusion of riboprotein granules and mitochondria may indicate the development of cytoplasmic streaming in a circular current around a fixed point. On the other hand, the more frequent occurrence of a dense core may point to hydration and delamination of a phospholipid body as demonstrated by Revel, Ito and Fawcett (8) as the source of the concentric pattern. The

possibility that the whorls are a form of Golgi apparatus cannot be excluded.

Since Revel, Fawcett and Ito, and subsequently Stoekenius, have produced concentric paired membranes using pure phospholipids and water and furthermore Stoekenius has demonstrated the effect of adding protein it seems safe to assume that myelin figures in cells are close to being pure phospholipide droplets. Questions to be answered by future research are the rate of their formation, the possibilities of reversibility and what alterations they cause in the overall activities of cells containing them.

Summary

Electron microscopic study of the endothelium in the dorsal root ganglia of old mice reveals two features not present in endothelium from young animals; namely, myelin figures and old age pigment. The association of the first named with the latter is suggestive that the occurrence of whorls of endoplasmic reticulum in endothelium is a feature of the aging process.

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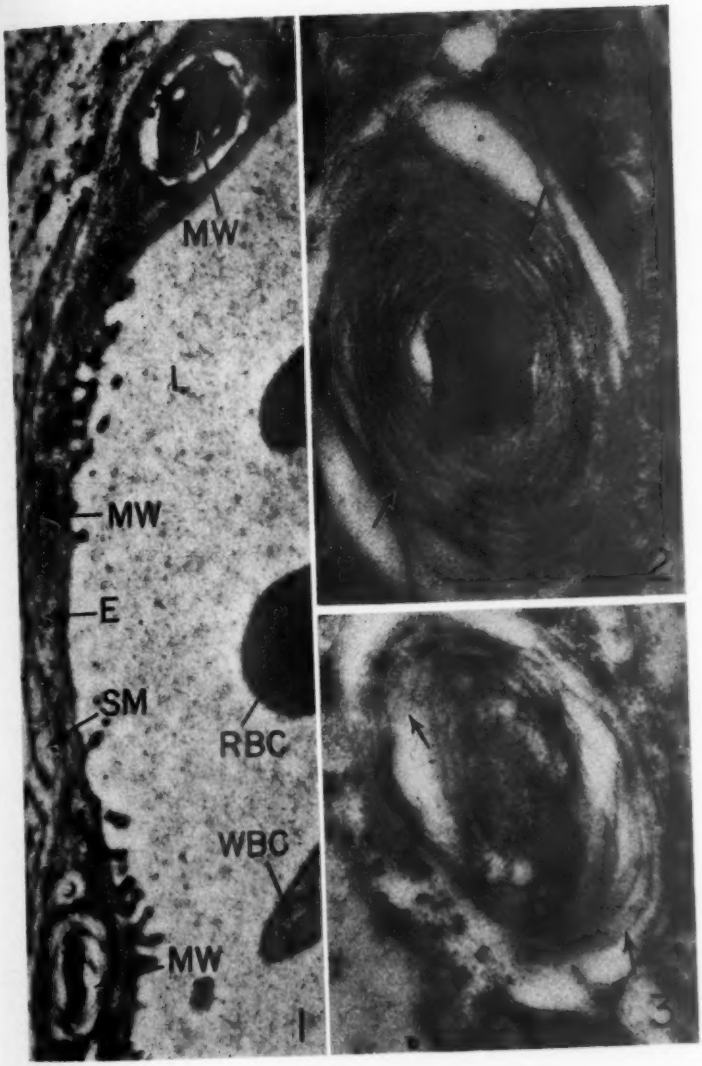
DESCRIPTION OF FIGURES

PLATE 1

FIG. 1. A section of a blood vessel with a wall of endothelium (E) and traces of smooth muscle (SM). Membranous whorls (MW) are a characteristic feature of the endothelium of capillaries, arterioles, and venues in the dorsal root ganglia of old mice. (L) lumen of vessel, (RBC) red blood corpuscle, (WBC) white blood corpuscle. x16,000.

FIG. 2. This figure illustrates the most frequent type of myelin body seen in endothelium. Notice the dense, structureless core, the pairing of concentric dense lines indicated by arrows, and irregular spaces between the paired membranes. x40,000.

FIG. 3. A small whorl with a core of cytoplasm containing ribonucleoprotein granules. Arrows point to individual membranes running obliquely through the section or ones that have been displaced from a normal to the section position by sublimation of the methacrylate. x40,000.


MW
L
MW
E
SM
RBC
WBC
MW
1
2
3

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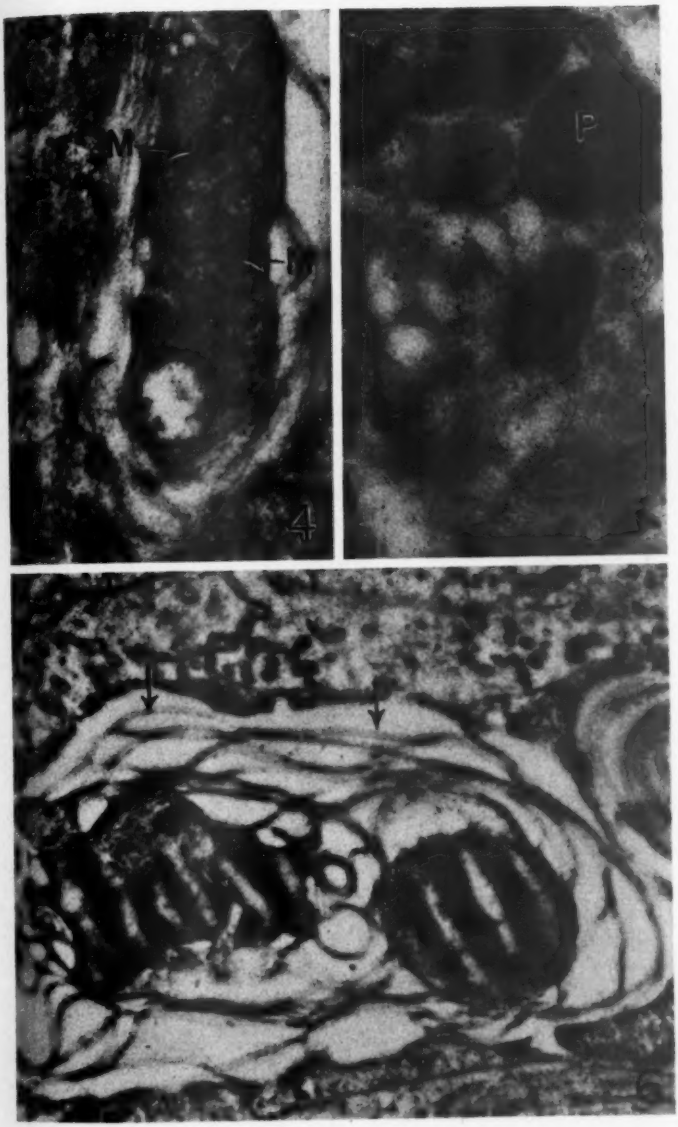
[2] NIH Experimental Training Grant Fellow.

PLATE 2

FIG. 4. A laminated body containing a core of mitochondria (M). x20,000.

FIG. 5. A bit of endothelium containing dense material (P) identical in appearance to the old age pigment found in adjacent neurons. x50,000.

FIG. 6. A large myelinoid body in the endothelium of a capillary. The membranous nature of the concentric lines is most apparent in places indicated by arrows. x18,000.


M
4
P

DETECTION OF METABOLIC ANTIGENS FROM NEMATODE LARVAE BY A MICROCULTURE-AGAR GEL TECHNIQUE*

LEROY J. OLSON, BARNA RICHARDS AND ADAM EWERT

The significance of the helminth metabolites in host-nematode relationships was first suggested by Sarles (1938). Chandler (1953) proposed that the formation of protective antibodies against the metabolic secretions and excretions of the invading helminth was an expression of an antienzyme-enzyme system of host immunity. Experimental support of this theory has been given by Thorson (1953) and others. While the exact nature of ES antigens (excretions and secretions) remains to be fully defined, some progress has been made in the chemical identification of helminth metabolites (Haskins and Oliver, 1958). This paper reports preliminary results obtained with a microculture-agar gel assay of larval ES antigens with *Trichinella spiralis* as a test organism.

Materials and Methods

The stock culture of *Trichinella spiralis* was maintained by passage of 2,000 infective larvae in 200 g male white rats every 6 to 12 weeks. Larvae were obtained from these rats by grinding infected skeletal muscle in a blendor for 20 seconds followed by digestion in 0.75% pepsin at pH 1.5 for 1 hour in a 37°C. water bath with continuous stirring. After recovery and washings in saline, larvae were suspended in nutrient broth-gelatin (6.4 g nutrient broth, 40 g gelatin, 1 liter distilled HOH) for determination of larvae per ml following microscopic examination of samples. Desired numbers of larvae were spun out, washed and placed in the following culture solution: Simms solution (2 parts)—beef serum ultrafiltrate (1 part); 500 units of penicillin and 500 micrograms of streptomycin were added per ml of culture solution (Campbell, 1955). Larvae were maintained at 37°C. throughout the above procedures.

ES antigen. Cotton-stoppered 50 ml. flasks each containing 25,000 larvae in 10 ml. culture solution were incubated at 37°C. for 5 days (Campbell, 1955). Lots in which the death rate of larvae exceeded 50% were discarded; internal disintegration and loss of motility were criteria of decreased viability and death.

Sonicated antigen. Lots of 10 ml. containing 10,000 larvae per ml. culture solution were placed in a Raytheon 220 W, 10 KC Magnetostrictive oscillator

for 30 min. A complete break-up of larvae was obtained. Sonicate and ES antigens were stored at —15°C. until used.

Immunization of rabbits. White rabbits were infected with 10,000 viable *Trichinella* larvae given orally in a nutrient broth-gelatin suspension at 3–4 week intervals. Blood from 6 infected and 6 control animals was taken by intracardiac puncture after the third immunizing infection, and monthly thereafter as needed. Sera were stored at —15°C until used.

Micro agar gel technique. A modification of Wadsworth's (1957) technique was designed to fit these studies. A plexiglass matrix (40 × 40 × 6 mm) with several funnel-shaped wells to hold the reactants was placed over a thin layer of agar gel. Seven wells, each with a capacity of 0.03 ml., were drilled with the top and bottom openings measuring 4.5 and 1 mm in diameter, respectively (Fig. 1). Approximately 0.6 ml. of 0.75% Nobles agar in 0.85% saline was poured on a heated glass slide immediately prior to placing the matrix in position. Thin strips of supporting tape were placed on the glass slide to retain a uniformly thin layer of agar between the matrix and glass slide. All slides were held 5 days at room temperature (22°C.) in a plexiglass chamber which minimized evaporation of reactants (Fig. 2). Semi-permanent mounts were made by immersing the preparation in cool water to loosen the plastic matrix, washing the exposed agar gel with saline and wax-sealing a glass cover-slip over the agar gel.

Results

Living *Trichinella* larvae were placed in the wells of the plastic and their metabolites allowed to diffuse down into the agar and

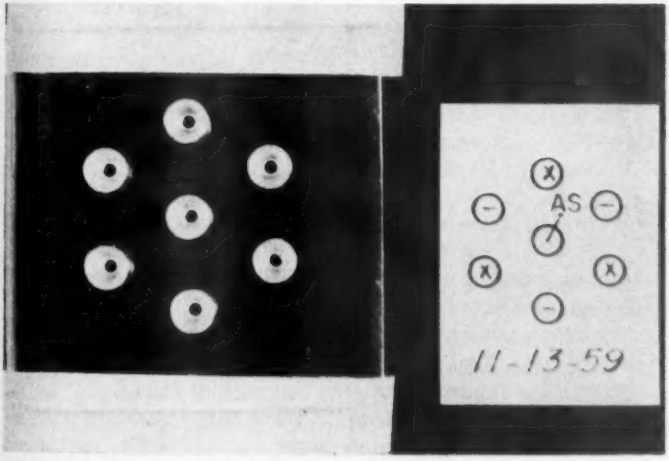


FIG. 1. Micro agar gel slide.

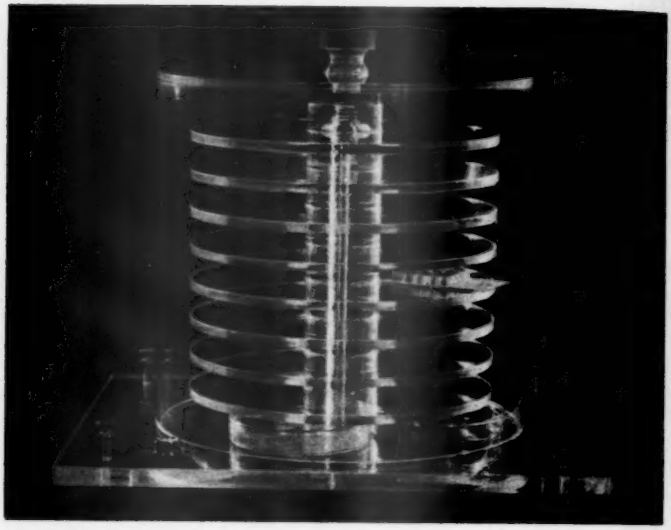


FIG. 2. Plexiglass chamber for storage of micro agar gel slides.

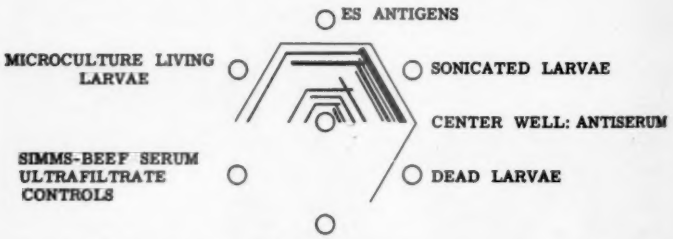


FIG. 3. Drawing of precipitation zones noted with micro agar gel technique for *Trichinella* antigens and rabbit antisera.

react with homologous antiserum diffusing from adjacent wells. Different numbers of larvae and dilutions of antiserum were tried to obtain optimal concentrations. Approximately 200 larvae per well seemed optimal for this system. Bands began to form in 48 hrs. with a maximum number of 4 noted on the third day (Fig. 3). Larvae survived well for the first 48 hrs., but on the third through the fifth day increasing numbers became non-motile with signs of international disintegration.

In control wells filled with freeze-killed larvae carefully washed before use and the above antiserum, a single band was seen which formed at 72 hrs. and coincided with one of the bands noted in the living preparation above (Fig. 3). Control wells with ES antigens (from 5 day cultures of living larvae) and the above antiserum showed a maximum of 6 bands which formed within 24 hrs. (Fig. 3). The 4 bands with living larvae appeared to show complete coalescence with bands in the ES antigen-antibody reactions. In studies with sonicated larval antigens and the above antiserum, a maximum of 9 bands were visible within 17 to 24 hrs. Four of these 9 showed partial or complete coalescence of zones with ES bands. Control wells with normal rabbit sera or culture solution were negative against the above antigens and antisera.

Discussion

These preliminary studies indicate that a microculture of nematode larvae in contact with an agar gel diffusion system is a workable method of assaying for metabolic products which are antigenic. Apparently both metabolic antigens and antigens comparable to those derived by sonication of larvae are released in the tissues of rabbits infected with living larvae. Studies with sonicate antigens indicate that at least 9 different antigens are released during a natural infection presumably as a result of the death and disintegration of larvae while in deep tissue locations and the release of a variety of antigens other than those associated with the living parasite. It also appears that 3 to 5 metabolic antigens (ES antigens) are present during an experimental infection as a result of the secretions and excretions of the living parasite *in situ*. The absence or partiality of coalescence noted for ES and sonicate bands suggests that a total of 13 *Trichinella* antigens are released during infection. Other workers have also noted the complexity of antigenic organization in *Trichinella*. Wodehouse (1956) found 10 precipitating antigens following chemical fractionation of larvae and injection of these materials into rabbits. Labzoffsky *et al* (1959) have found 7 antigens by chemical fractionation of larvae and infection of rabbits with living larvae; these workers also noted that circulating antibodies against these fractions appeared at different times following the initial exposure of the host to larvae.It is possible that with more refined techniques, addi-

tional antigens will be detected for this metazoan parasite. In related studies (Ewert and Olson, 1959) a comparison of immunogenicity in mice was made for ES and sonicate antigens of *Trichinella*. A significant protection against a challenge infection of living larvae was noted only in mice previously injected with ES antigen (or sensitized with living larvae); mice injected with sonicate antigens showed no significant resistance to a similar challenge as compared to ES or control injected mice.

The difference in time of appearance and band size for the antigens tested may depend on the preparation. ES antigens produced visible bands in less time as compared to similar bands resulting from microculture of larvae, since the latter wells lack ES antigens at the time of charging and must depend on the larvae present to produce these antigens. Wells charged with sonicated material also have immediately diffusible antigens; the presence of ES antigens within the sonicated material presumably reflects ES antigen present but unreleased in the nematode body prior to sonication.

This technique is now being applied to the characterization of antigens from living *Toxocara canis* larvae and the serology of human visceral larva migrans. Several bands have been detected when microcultures of *Toxocara* larvae were run against sera from experimentally infected rabbits or suspected human cases of this infection. Intra-gel absorption is also being evaluated with this *Trichinella*-micro agar gel system for application to the problem of cross reactions between *Ascaris lumbricoides* and *Toxocara* antigen-antibody systems. Results with *Toxocara* antigens will be reported elsewhere.

Summary

Microcultures of *Trichinella spiralis* larvae have been incorporated into a micro agar gel double diffusion technique with the result that the excretions and secretions of living larvae are allowed to diffuse directly into the agar gel and react with homologous rabbit antiserum to produce several visible bands of precipitate. Bands from these microcultures were compared to bands from sonicated larvae, dead larvae and nematode metabolic products from previous cultures of larvae.

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A COMPARATIVE STUDY OF THE EFFECTS OF SUCCINYLCHOLINE ON VERTEBRATES[a]

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Succinylcholine chloride, a short-acting skeletal muscle relaxant, causes muscular paralysis in mammals by producing a blockage of nervous transmission at the myoneural junction. This blockage apparently depends on a depolarization of the muscle end-plate membrane. The short duration of the drug action in mammals is due to its rapid hydrolysis into succinate and choline by the "pseudo-cholinesterase" of the plasma (20).

Since the original description of the pharmacologic action of succinylcholine (3), there have been many suggestions for its clinical application. Probably most important among these has been the production of muscle relaxation during various surgical procedures (6,9,10,14,16,23,24,28) and in conjunction with electroshock therapy (10,17,28,30).

The physiological effects of this drug cannot be generalized and variations have been demonstrated between species, and within the same species, depending upon the muscle or muscle groups under consideration. The first effect of the drug on man and many mammals is an augmented muscle contraction and twitching, followed by a gradual relaxation until no muscle response is obtained on stimulation of the nerve. One exception to this pattern of relaxation is the extraocular muscles, which contract and possibly cause the increased intraocular pressure associated with the use of this drug (12,19). Several investigators (5,8,29) have described similar effects of succinylcholine on laboratory animals such as the cat, rabbit, and mouse.

Some unusual effects of this drug have been reported for other laboratory animals. Bovet (4) noted a contracture, similar to a nicotinic or acetylcholine contraction, in the rectus abdominis muscle of the frog, instead of the expected relaxation. In birds, there is contracture of muscles and convulsion following the administration of "leptocurare" drugs (decamethonium, succinylcholine) (3,7). Recent experiments with urodeles (22) show that succinylcholine chloride does not relax their muscles, but to the contrary, causes a spastic paralysis of the entire body.

These results, which demonstrate that the effects of succinylcholine vary tremendously among different species, indicated that an attempt should be made to obtain additional information on the effects of this drug on various species. The purpose of this project is to report preliminary results on representatives of the vertebrate classes (amphibia, reptiles, birds and mammals).

Materials and Methods

The following representatives of the vertebrate classes were selected:

Mouse, *Mus musculus*
White Rock chicks, *Gallus domesticus*
American chameleon, *Anolis carolinensis*
Leopard frog, *Rana pipiens*
Common spotted newt, *Diemictylus viridescens*

The stock solution of succinylcholine chloride ("Anectine" chloride brand, Burroughs Wellcome and Company, Inc., Tuckahoe, N.Y.) contained 20 mg of the drug per milliliter. Because of the small weight of many of the experimental animals, a dilution of the commercial preparation was utilized to assure a more accurate injectable quantity. The dilution was prepared by adding 0.5 ml of the stock solution to 100 ml distilled water (0.1 mg/ml). No apparent difference was observed in the effect of a given dose of either the diluted or commercial preparation.

Initially, the dosages utilized were determined empirically, but once established, they were administered in amounts proportional to the body weight of the animal (mg/kg).

In all cases the drug was given intraperitoneally. This route of injection was selected because of the difficulty, and sometime impossibility, of giving intravenous injections to animals as small as the newt.

No anesthetic or other drugs were used or tested in conjunction with succinylcholine. All experiments were conducted at room temperature (22–23°C).

No attempt was made to determine the LD_{50} of the drug for each species. However, the minimal immobilizing dose was noted and hereafter will be referred to as the "MID". The MID is defined as the minimal dose of the drug in mg/kg which paralyzes an animal to the extent that it has no "righting reflex" response.

Results

The response to a dose of succinylcholine chloride given intraperitoneally varied greatly both within each class and among all the classes of the vertebrates tested. The minimal immobilizing dose (MID) for each class is summarized in Table 1.

MOUSE: The 8 mice that were used had an average weight of 31.3 grams (range 23.0–41.0 g). Animals were immobilized with a flaccid paralysis for less than 10 minutes with doses of

TABLE 1

Minimal immobilizing doses of succinylcholine

Animal	mg/Kg
Mouse	1.50
Chick	0.38
Lizard	0.75
Frog	1.50
Newt	9.00

1.5 mg/kg (MID). One mg/kg of the drug had no apparent effect on the mice, while doses exceeding the MID resulted in death. The dying animals had reduced respiratory movements and were characterized by increasing cyanosis prior to death. No attempt was made to save these animals by use of a stimulant or a respirator. All groups of muscles appeared to be relaxed by the MID or by lethal doses. No spastic paralysis was observed among these animals during the course of the experiments.

CHICK: Two groups of chicks were used during the tests. One group of 8 (1 week old) had an average weight of 37.7 g (range 34.5–46.7 g); the second group of 7 (2 weeks old) had an average weight of 62.5 g (range 55.0–66.0 g). The initial dose tried on a chick was 1.5 mg/kg succinylcholine (mouse MID), which resulted in immediate spastic paralysis and death of the animal. The paralysis was characterized by a hyperextension of the legs and toes and opisthotonos (Fig. 1). This paralysis also was observed in animals receiving smaller immobilizing doses which were non-lethal. During the recovery period chicks showed some movement of the wings, which apparently were not affected by the drug. The recovery period for the MID ranged from 24 minutes to 2 hours. The MID for chicks of both groups was 0.375 mg/kg (or ¼ mouse MID). When the dose was increased to 0.56 mg/kg, death resulted probably as a consequence of relaxation of the intercostal muscles (hypo-ventilation).

LIZARD: Ten lizards (adult chameleons) had an average weight of 4.1 g (range 3.5–5.4 g). One of these animals was immobilized and unable to "right" for approximately 1 hour with a dose of 0.375 mg/kg. Two animals given 0.75 mg/kg remained immobilized for 103 and 135 minutes, respectively. Of the 4 animals given 1.5 mg/kg succinylcholine (mouse MID), two were

FIG. 1. Characteristic paralysis of chicks resulting from succinylcholine chloride intoxication.

able to right after 187 minutes, and one after 207 minutes; one of these 4 was immobilized for approximately 8 hours. Animals receiving an immobilizing dose displayed a definite pattern of paralysis. The entire body became rigid in 1 to 3 minutes postinjection; frequently, the forelimbs showed hyperextension subsequent to a hyperflexion of the hindlimbs. This pattern of hyperextension of the forelimbs and hyperflexion of the hindlimbs was consistent among the immobilized animals. The spastic paralysis gradually subsided during the recovery period until the animal showed a relaxation not dissimilar to that produced by a general anesthetic (e.g., ether). Frequently, during the spastic paralysis, kyphosis was observed.

In an attempt to determine the minimal lethal dose for these animals interesting phenomena were observed. The chameleon was able to tolerate doses of succinylcholine as high as 64.0 mg/kg. With doses ranging from 3.0 to 64 mg/kg, animals underwent a short period of overall rigidity of approximately 30 seconds duration, followed by a longer period of relaxation proportional to the dosage (approximately 18 hours for 64 mg/kg). During this period the axial musculature was relaxed as in gen-

eral anesthesia while the limb musculature appeared to retain its normal tone. These phenomena are inexplicable in the light of the foregoing results.

FROGS: Seven frogs had an average weight of 48.4 g (range 41.0–58.0 g). The MID for these animals was 1.5 mg/kg succinylcholine (= mouse MID). This dose produced an immobilization characterized by inability to right, spastic paralysis of the forelimbs, and a flaccid paralysis of the hindlimbs. These symptoms persisted for approximately 1½ to 2 hours. Recovery was marked by a return of tone to the hindlimbs, a relaxation to normal tone for the forelimbs, and a righting of the animal. Frogs showed no effects from 1.0 mg/kg succinylcholine; they survived a dose of 2.25 mg/kg, but died after injection of 3.0 and 6.0 mg/kg. Death, however, did not occur until approximately 60 hours postinjection.

NEWTS: The group of 14 adult newts had an average weight of 2.4 g (range 1.5–3.0 g). Doses of succinylcholine up to 6.0 mg/kg did not immobilize these animals, although some effects were noted. Frequently, at lower doses (e.g., 1.5 mg/kg), the animals walked with a stumbling gait, dragging their limbs. A dose of 6.0 mg/kg suppressed the righting response, but did not prevent a response to a painful stimulus. The MID for newts was 9.0 mg/kg succinylcholine which immobilized them for approximately 2 hours. Twelve mg/kg immobilizeds these animals for 24 hours. Newts proved very resistant to the lethal effects of the drug and survived doses greater than 100X the mouse MID (160 mg/kg). Animals receiving doses of this magnitude developed extreme edema of the entire body during the first 24 hours of immobilization, but they gradually returned to normal during the recovery period. Another outstanding characteristic of injected newts was a complete spastic paralysis of the body occurring within 5 minutes postinjection (Fig. 2). As in the lizards, the forelimbs were hyperextended and the hindlimbs were hyperflexed.

During the recovery period injected newts were maintained at 22° C. in a constant temperature room.

Similar experiments conducted at higher temperatures (31–33° C) with acclimatized newts and lizards gave results which corresponded with those described above, thereby indicating that

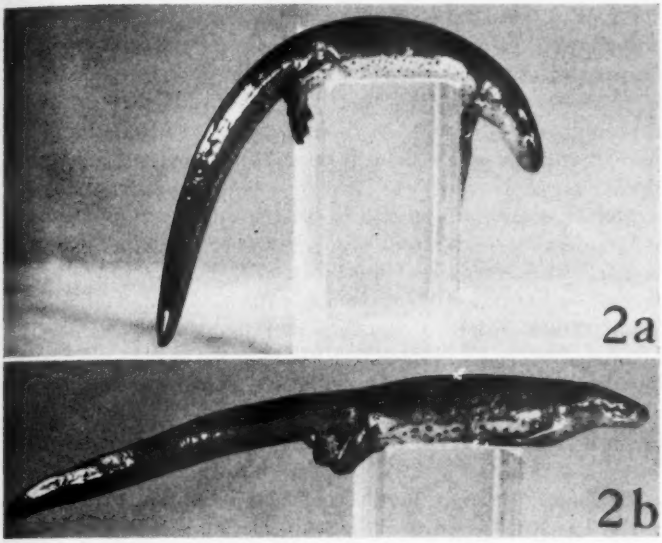

FIG. 2a. Newt anesthetized with tricaine methanesulfonate (MS-222, Sandoz), a general anesthetic for amphibians.
FIG. 2b. Spastic paralysis of newt induced by I.P. injection of succinylcholine chloride.

the unusual effects of the drug on poikilotherms were not dependent on a lower body temperature and metabolic rate.

Preliminary injections of succinylcholine chloride have been given to several species of fish, and these results will be reported at the termination of the project.

Discussion

Different classes of vertebrates showed a w le variation in their response to injections of succinylcholine chloride. The mice responded to the drug in the same manner as described for other mammals (including man). The drug acted as a rapid muscle relaxant with an effect of short duration. The minimal immobilizing dose determined by intraperitoneal injections differed somewhat from reported I.V. doses. For example, de Beer and his associates (8, 31) found that 0.05 mg/kg given intravenously in cats was effective in producing muscle relaxation. The intraven-

ous L. $D._{50}$ for albino mice was reported as 0.55–0.59 mg/kg (Burroughs Wellcome Laboratories). Generalized muscular relaxation in the adult human followed the I.V. injection of 0.3 mg/kg (19) and 0.42 mg/kg (30) of succinylcholine. The difference in the I.P. and I.V. doses was expected because of the slower entrance of the drug from the coelom into the vascular channels.

The most unusual differences between the mammals and the submammalian vertebrates were (1) the development of a spastic paralysis in the lower forms, instead of the expected muscular relaxation, and (2) the prolonged action of the drug without lethal effects, instead of the short duration usually observed in mammals. The only mammalian muscles reported to contract following injections of succinylcholine are the extraocular muscles, which have been studied both *in vitro* (12) and *in vivo* (12, 19), because of the observed effects of the drug on intraocular pressure.

The results reported here on chicks were similar to those previously reported. Drill (13) stated that birds exhibited spastic paralysis of skeletal muscles and opisthotonos when injected with "depolarizing" curareform agents. Decamethonium iodine and succinylcholine caused spasticity (7) and convulsion (nicotinic action) in birds (4). Succinylcholine did not affect all muscle groups equally; the muscles of the legs and neck contracted, but the wings retained their normal tone (or in some cases appeared relaxed). Frequently, when the legs were still rigid the wings moved as the animals attempted to stand.

The two species of Amphibia exhibited a different response to the drug. The newts, which are considered more primitive phylogenetically than the specialized frog, became completely rigid after injection of the drug. This type of spastic paralysis was noted only in the forelimbs of the frog; the hindlimbs were flaccidly paralyzed. The rectus abdominis muscle of the frog was stimulated to contract following treatment with decamethonium (25), a drug which resembles succinylcholine in its action. Other curareform drugs, such as curare (18) and d-tubocurarine (21) inhibited the contraction of the frog rectus abdominis muscle. These differences in muscle group response to the drugs emphasize the caution which should be practiced in using amphibian

nerve-muscle preparations for assay of drugs without first running extensive controls.

The chemical structure of succinylcholine, which has been described simply as two molecules of acetylcholine linked together at the alpha-methyl groups, may explain the strange effects of the drug on lower vertebrates. Normally, in mammals, upon injection into the blood stream, succinylcholine is rapidly hydrolyzed by nonspecific plasma cholinesterases (= pseudocholinesterases) into succinylmonocholine, which also has neuromuscular blocking action. This compound then is hydrolyzed into succinate and choline which do not have this action (15). Drill (13) proposed that in mammals succinylcholine maintained a depolarization of the nerve end plate, thereby blocking neuromuscular transmission. This evidently does not explain the action of this drug in lower vertebrates. In these forms there is possibly a competition between the normally occurring acetylcholine and the injected succinylcholine for the cholinesterases. This competition may cause concentrations of ACh equal to or higher than normal to be present at the synapse, resulting in a continuous neuromuscular transmission. The injected succinylcholine chloride may have the same capacity as ACh in producing muscular contraction; however, acetylcholinesterase may not be able to hydrolyze succinylcholine (into ineffective products) as effectively as ACh. If a competition does exist, and the cholinesterase is depleted temporarily, this may explain the prolonged effect of the drug on lower forms also. Davis et al. (11) suggested that the prolonged effects of succinylcholine in hypercapnic dogs was due to a lower concentration of potassium in the muscle. Potassium antagonizes the action of curare and succinylcholine (11). When hypercapnia exists the myoneural apparatus is rendered more susceptible than usual to the effects of drugs which produce muscular paralysis. Since there are such variations in the action of the drug, this proposal probably does not apply to lower vertebrates; however, the hypercapnia present in animals that are immobilized for long periods certainly may have affected their neuromuscular response to the drug.

Another possible explanation for these unusual responses to succinylcholine may be related to the fact that there are different cholinesterases in various species. There are at least two types of

cholinesterases, a "pseudocholinesterase" found in mammalian serum, and an acetylcholinesterase found in all excitable tissues (26) which shows a sharp optimal substrate concentration (1, 2). Pseudocholinesterase is important in the hydrolysis of succinylcholine in mammals (see references above) and if it is not present in the plasma of lower animals, this might explain the prolonged effect of the drug.

Quarternary ammonium base compounds have been claimed to be strong inhibitors of acetylcholinesterase (27). Succinylcholine has this type of chemical structure; therefore, it may inhibit the action of acetylcholinesterase, thereby affecting the repolarization at the neuromuscular junction.

Summary

1. The effect of succinylcholine chloride, a short term muscle relaxant, on representatives of the vertebrate classes was investigated.

2. This drug induced general muscular relaxation in mice, as in other mammals and man. In contrast to its effect on mammals, succinylcholine caused different degrees of spastic muscular paralysis in chicks, lizards, frogs and newts.

3. The possible modes of action of this drug on submammalian vertebrates were discussed.

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SILICONE TREATED SUTURES IN SMALL ARTERY ANASTOMOSIS

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Vascular grafting procedures and other arterial operations sometimes fail because thromboses form at the line of anastomosis of small blood vessels (2, 4, 5, 10, 14, 15). The use of conventional suture materials, which are highly absorptive, may increase the chances of such thromboses occurring, since many investigators have reported that absorptive foreign material in the body increases the blood's ability to clot (4, 7, 6, 20), and since Tocantins (20) has shown that such absorptive material reduces the amount of clot-inhibiting substances in the blood. By performing femoral artery transection and repair in dogs, we have tried to determine the value of silcone-treated stutures in preventing such anastamotic throboses.

Materials and Methods

A ten-minute soaking in a silicone solution (dimethyl polysiloxane) (1) rendered arterial sutures non-wettable. The sutures† (braided 5–0 silk on non-traumatic needles) were washed for five minutes in running water to remove solvents present in the treating solution and were then autoclaved. We shall hereafter refer to these sutures as the "treated sutures." The "control sutures" differed only in that they had not been treated with silicone.

Twenty adult mongrel dogs were anesthetized with Nembutal.‡ With aseptic surgical technique, both femoral arteries were exposed and the loose connective tissue carefully dissected away from the region of the proposed transection and repair. The same person performed all operations, using a consistent technique. Occluding tapes were placed one centimeter above and below the cleared areas, and the two arteries were divided obliquely, at approximately a 45-degree angle. They were then biangulated with 5–0 stay sutures and repaired by the over-and-over continuous suture technique. Control sutures were used in the right arteries, and treated sutures in the left arteries. Great care was taken to avoid the creation of a stricture at the anastomosis. The vessels were kept moistened with isotonic saline during exposure. No anticoagulants were used. Because of the wounds' proximity to contaminated areas and the animals' habit of licking their wounds, all dogs received penicillin and streptomycin for 5 days postoperatively to prevent thrombosis due to wound infection. After a follow-up period of 58–98 days, the anastomotic areas of the arteries were removed for histologic study.

Results

Table I lists the length of follow-up time in each dog and presents data obtained at re-operation: the state of patency of the anastomoses and measurements of the outside diameters of the vessels, which ranged from 2.0 to 3.8 mm. Data from 5 dogs are excluded, because in these animals a wound infection occurred or an anastomotic stricture was evident during the original operation.

No artery repaired with treated sutures became thrombotic, but in one such artery, a postoperative stricture developed. The vessel remained patent, however. In the arteries repaired with control sutures, there were no strictures at re-operation, but in two of these arteries, histologic studies revealed an organized thrombus extending from the suture line (Figure 1).

In all arteries, the treated and the control sutures which were exposed to blood flow had been covered by the time of re-operation. with a thin layer of endothelium.

The animals differed from each other in the extent of their

TABLE I

No.	Untreated	Treated	Outside Diameter	Follow-up	Remarks
1	patent	patent	2.0 mm.	90 days	
2	patent	patent	3.0 mm.	80 days	
3	patent	patent	2.3 mm.	90 days	
4	patent	patent	3.0 mm.	98 days	
5	patent	patent	3.0mm.	95 days	
6	patent	patent	3.0 mm.	94 days	
7	patent	patent	3.8 mm.	68 days	
9	patent	patent	2.5 mm.	80 days	
11	patent	patent	3.0 mm.	76 days	
12	patent	patent	3.0mm.	70 days	Tear in experimental side (treated)-required additional stitch for repair.
14	patent	patent	3.5 mm.	59 days	
15	patent	patent	3.0 mm.	77 days	
16	patent	patent	3.0mm.	77 days	Tear in experimental side (treated)-required additional stitch for repair.
17	thrombosed	patent	3.0 mm.	77 days	Each side required additional stitch (one) for hemorrhage.
18	thrombosed	patent	2.0 mm.	77 days	Treated side required an additional suture for hemorrhage.

reaction to foreign bodies. Each dog's reaction to the treated sutures, however, was the same as his reaction to the control sutures.

Discussion

Sawyer *et al* (16) have reported that during the immediate post-anastomotic period, abnormal electrical potentials present in the walls of transected arteries predispose the vessels to thrombosis. It is likely that during this period, conventional sutures contribute to such a thrombosis. Within 3 to 7 days after operation, however, the suture line in small-artery anastomosis is generally covered with a layer of endothelium (5, 14). No techniques were available to determine whether any of the silicone remained on the sutures at the time of re-operation, but it is doubtful that, after covering by endothelium, the presence or absence of silicone on the sutures has an effect in preventing thrombosis. At that time, the patency of the vessel probably depended upon the presence of a stricture-free anastomosis and an adequate blood flow across the anastomotic line (6, 11, 15, 17).

The incidence of thrombosis in the vessels anastomosed with control sutures in this experiment corresponds with results of previous work in animals and human beings (2, 4, 10). The comparative results between the two types of sutures are suggestive that siliconized sutures will reduce the formation of thrombi but because of the small number of animals in the series the results are not statistically significant.

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‡ Courtesy of Abbott Laboratories.

THE EFFECT OF METHYLCHOLANTHRENE ON THE GASTRO-INTESTINAL TRACT OF THE DUCK

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The neoplastic effect of methylcholanthrene has been observed in a wide variety of hosts and in many tissues (9, 41). Among the hosts studied are: cockroaches (37), frogs (36), salamanders (2), newts (3, 15), ducks (4), fowls (4, 12, 22, 23, 33, 45), pigeons (4, 5), guinea pigs (10, 34, 40), monkeys (6, 14, 24, 25), rabbits (1, 8, 11, 42, 43), cats (7, 39), dogs (7, 39, 43), turkeys (4, 31), hamsters (13, 21, 35) and, of course, mice and rats. The tissues in which methylcholanthrene has been put include the skin, subcutaneous tissue and muscle. It has been put into the serous cavities and injected into most of the viscera, lymphoid tissues, bones, brain, endocrine and salivary glands; also, it has been given intravenously (9). Apparently few investigators have been interested experimentally in the effect of methylcholanthrene on a variety of tissues in the same host.

In reviewing this problem of the effects of methylcholanthrene on different hosts and different tissues, no attempt has been made to evaluate the fact that some investigators have been able to produce neoplasms in an animal where others have been unsuccessful. This summary of our experience with methylcholanthrene in the duck is made only to emphasize that variations in the occurrence of tumors do occur in different tissues in a single host. This fact alone should point out the necessity for an experimental approach to the problem of carcinogenesis in each host and in each tissue, rather than accepting the philosophy that a known carcinogen will produce a neoplasm regardless of the host and the tissue.

During the past 10 years we have been interested in the neoplastic response of the white Pekin duck to methylcholanthrene. During this time methylcholanthrene has been given in the following manner: 1. put directly into the muscle and into the trachea; 2. suspended in mineral oil and injected intramuscularly and given orally and intratracheally; 3. given orally with wheat flour in gelatin capsules; 4. suspended in sesame oil and given

intramuscularly; 5. dissolved in acetone and applied locally to the skin; 6. suspended in polysorbate 80. U.S.P. (Tween 80) and given intratracheally; 7. compressed into pellets and given intracerebrally, intramuscularly, subcutaneously and intraperitoneally.

A wide variety of neoplasms has occurred in the skin following the local application of methylcholanthrene in acetone. Among these tumors have been hemangiomas, fibromas, neurofibromas, ganglioneuromas, papillomas and squamous cell carcinomas (26, 27, 28, 29). It is of interest to observe that the tumors occurring in the skin of the thoracic wall differ from the type of lesions developing in the skin of the web of the foot and in the skin beneath the lower bill (30).

Hemangiomas, neurogenic tumors and sarcomas have developed in the pectoral muscle following the local injection of methylcholanthrene in both mineral and sesame oil and only with the crystals (26, 28). Methylcholanthrene crystals put directly through the external larynx into the first portion of the trachea have resulted in squamous metaplasia and a questionable squamous cell carcinoma (32). Only a few birds have been given the crystals directly into the trachea because of technical difficulties. Methylcholanthrene suspended in mineral oil, when put into the trachea through the external larynx, has produced a papilloma of the mucosa of the trachea and proliferation of fibrous tissue in wall of the trachea (32). The latter was difficult to evaluate either as a neoplasm or as an inflammatory process. Methylcholanthrene suspended in polysorbate 80, U.S.P. (Tween 80) and put directly into the trachea through the external larynx has resulted in the development of a large number of neoplasms, both carcinomas and sarcomas (32).

Pellets of methylcholanthrene prepared by packing the crystals into a compact mass (4x2 mm; wt. 4–8 mg) and then injected through a trochar into the brain of 49 ducks have not resulted in the occurrence of a neoplasm. The pellets were present in the brain of some of these ducks for 370 days. Only one hemangioma occurred in the pectoral muscle of one duck on the 106th day in a group of 20 following the implantation of methylcholanthrene pellets. Some of the pellets were present in the muscle after 338 days. No pellets were recovered from the subcutaneous tissue after the 22nd experimental day.

In many of these experiments it has been impossible to ascertain the interval between the injection of the carcinogen and the time in which the neoplasm began to develop. Tumors have been observed in the respiratory tract within 150 days after the intratracheal injection of methylcholanthrene (32). Hemangiomas and papillomas have occurred in the skin of the body 60 days after the first of 30 local applications of methylcholanthrene dissolved in acetone (29). These observations do show, however, that neoplasms may occur in the skin and in the respiratory tract after relatively short intervals. We have been unable to form any opinion from these experiments referable to the longest interval between the application of methylcholanthrene and the development of a neoplasm. Furthermore, no attempt has been made to study the effect of the vehicle on the time of the appearance of neoplasms.

To observe the tumor response of ducks to the oral administration of methylcholanthrene, 21 birds 19 days of age were given 7.5 mg. of methylcholanthrene suspended in 1.0 ml. of liquid petrolatum (mineral oil) daily for 30 days. These injections were given five days each week. The ducks received a total of 225 mg. of methylcholanthrene. Four of the ducks died on the 287th, 364th, 382nd and 485th day following the first treatment. Fourteen of the ducks were killed and examined on the 487th day, as shown in Table 1. At

TABLE 1

Ducks given methylcholanthrene orally

No. ducks	Age/days	Amt./mg.	Total no. doses	Autopsied	
				No.	Age/days
21	19	7.5 q.d.[1]	30	2	91–98
				2	387–298
				2	365–382
				15	485–487
3	125	30 q.d.[2]	20	1	49
				1	120
				1	343
5	48	30 b.i.d.	20	1	49
				4	421
1	75	30 b.i.d.	20		28
4[3]	75	30 b.i.d.	20	1	141
				3	392

[1] 7.5 mg. methylcholanthrene per ml. in mineral oil.
[2] 30 mg. methylcholanthrene in 270 mg. flour in gelatin capsule.
[3] Twenty-four days later these ducks were given an additional dose of 30 mg. q.d. for 20 days.

the time of autopsy the ducks given methylcholanthrene in mineral oil did not have a gross tumor or anything even suggesting a neoplastic lesion in the intestinal tract.

A second group of 13 ducks, as shown in Table 1 were given 30 mg. of methylcholanthrene crystals mixed with 270 mg. of flour. This mixture was put into a gelatin capsule and placed directly into the esophagus. Three of these ducks were given a capsule once a day for 20 days, six birds were given a capsule twice a day for 20 days, and four were given 30 mg. of methylcholanthrene twice a day for 20 days, and 24 days after completing this experiment they were given an additional 30 mg. of methylcholanthrene once a day for 20 days. The first group of 3 ducks received 600 mg. of methylcholanthrene during the course of the experiment, the second group of six received 1200 mg. and the third group of four received a total of 1800 mg. Table 1 shows the time when these ducks were killed. Eight of the 13 ducks were sacrificed either on the 343rd day or later. None of the ducks showed a gross neoplasm or anything to even suggest such a lesion.

The gastro-intestinal mucosa was observed for macroscopic fluorescence in five ducks using an ultraviolet lamp (Aloe; No. 52140 ultraviolet, mineralight, high-intensity, long wave, 3, 66A). The intestinal tract was opened and examined immediately after the birds were killed. Portions of the contents of the colon and the stools were placed on slides and examined microscopically using a Zeiss fluorescence microscope with a mercury vapour lamp OSRAM HBO 200.

There was no macroscopic fluorescence of the mucosa of a normal duck. Fluorescent particles, however, were present microscopically in the intestinal contents and in the stools of a normal duck but nothing was observed to suggest methylcholanthrene crystals. The intestinal contents of four ducks, given 30 mg. of methylcholanthrene crystals in a capsule with 270 mg. flour and killed either three or six hours later, did not show any macroscopic fluorescence suggestive of methylcholanthrene. The contents of the colon, likewise, did not show microscopically any methylcholanthrene crystals. Methylcholanthrene crystals were present microscopically, however, in the stools from these ducks.

Obviously, methylcholanthrene crystals following oral administration are rapidly excreted into the feces of the duck. A duck of the same age and size was given 30 mg. of methylcholanthrene crystals in flour and the stools were examined for methylcholanthrene crystals during a period of two hours and forty-five minutes. A specimen of stool was obtained immediately before the methylcholanthrene was given and at the fol-

lowing intervals thereafter: 14, 35, 50, 70, 95, 105, 120, 135, 155 and 165 minutes. Methylcholanthrene crystals were found for the first time in the stool passed after two hours and 45 minutes. Subsequent stools also showed the crystals.

Discussion

We have been unable so far to obtain any tumors in the brain of ducks following implantation of methylcholanthrene pellets. Russell and Loquvam (33) also were unable to produce tumors in the brains of chickens with pellets composed of methylcholanthrene and cholesterol. Shear (39) did not obtain a tumor following the implantation of a methylcholanthrene pellet into the brain of a cat. Zimmerman (46) and Nakamuray (19), however, did produce gliomas in mice and rats when methylcholanthrene pellets were injected.

In the duck we have not observed any neoplasms following the oral administration of methylcholanthrene in liquid petrolatum and in wheat flour given as capsules. Mori et al (18) added methylcholanthrene to the stock diet of rats but did not observe any carcinomas. Shay and associates (38) gave methylcholanthrene in 0.5 ml. of olive oil to rats by stomach catheter but did not produce any tumors. One rat in a group of 18 studied by Mori et al (17) developed an adenocarcinoma of the colon following the oral administration of an emulsion with methylcholanthrene. From these observations it would seem that the rat is not prone to develop neoplasms in the gastro-intestinal tract following the oral administration of methylcholanthrene. Van Prohaska et al (44) fed mice methylcholanthrene and found benign papillomas of the fundus of the stomach of two in a group of 48 observed for 186 days. These investigators commented, "Although methylcholanthrene is chemically related to the bile acids, it does not exhibit a marked degree of potency on oral administration as regards induction of neoplasms even in the upper region of the alimentary canal, where the lining is stratified squamous epithelium." Nau et al (20) frequently observed squamous cell carcinomas in the forestomach of mice fed methylcholanthrene. However, when methylcholanthrene was absorbed on carbon black and fed to mice, it was ineffective as a carcinogen.

Obviously there are significant basic factors influencing the carcinogenic action of methylcholanthrene on the gastro-

intestinal tract of different hosts. In such studies it should be remembered that a carcinogenic agent may be changed by the intestinal secretions: it may be absorbed and rendered innocuous or it may not be absorbed by the mucosa. The present observations in the duck would suggest that the failure of tumors to develop in the intestinal tract following the oral administration of excessive amounts of methylcholanthrene is due to the rapid excretion of the crystals.

The failure to obtain neoplasms in the gastro-intestinal tract of ducks following oral administration of methylcholanthrene, although many neoplasms developed in the skin following local application of methylcholanthrene and in the respiratory tract following intratracheal inoculation, is interesting in view of the observations made by Kolas (13) on the oral mucosa of mice with methylcholanthrene. The palatal mucosa of mice subjected to topical applications of methylcholanthrene did not develop intraoral carcinomas, but malignancies did develop in the skin of the face where the carcinogen had spread. The mucosal surface of the lip of mice was refractory to 9, 10-dimethyl-1, 2 benzanthracene, while the adjacent skin of the lower lip revealed the characteristic changes resulting from this carcinogen (16). Experimental observations, such as these, with known carciongens in different tissues of the same host only emphasize the necessity of scientific caution in speculating on the neoplastic effect of a suspected carcinogen in a specific tissue of any host.

Summary

White Pekin ducks given methylcholanthrene orally, suspended in liquid petrolatum and crystals in a gelatin capsule with wheat flour, have not shown any tumors in the gastro-intestinal tract. Methylcholanthrene crystals are rapidly eliminated from the intestinal tract as shown by the ultraviolet examination of the mucosa and the feces. The absence of neoplasms may be explained by the failure of absorption of the carcinogen. This study emphasizes that neoplasms accepted on the basis of "association" with a carcinogenic agent always should be critically evaluated.

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A STUDY OF THE ROTARY COMPONENT OF THE MOTION OF THE ARYTENOID CARTILAGES IN MAN

WILLIAM W. SULLIVAN, MARY ELMORE SAUER AND GUNTER CORSSEN*

As long ago as 1855, Manuel Garcia (1) described the rotary motion of the arytenoid cartilages following indirect laryngoscopy of his own larynx. Subsequent observers and current teaching (2, 3, 4) have consistently attested to this rotary component in direct or indirect methods and descriptions. Their bases have been the morphology of the arytenoid-cricoid articulations and the laryngeal musculature as well as observations in living specimens of man and lower animals in comparative studies.

Moore and von Leden (5, 6) have recently made an excellent film of the larynx in action. Therein they allude to the pure "smooth lateral glide" motion of the arytenoid cartilages, denying any rotary component. The technic employed was indirect laryngoscopy without anesthesia. Since the major portion of the film dealt with the vocal folds in the process of phonation, the camera was centered on the folds.

Thorough scrutiny of their film has convinced us that the arytenoid cartilages are incompletely viewed. Also, we observe evidence of rotary motion taking place.

One of us (W.W.S.) submitted to a premedication of 75 mgs. Meperidine and 0.32 mgs. Scopolamine. One hour later, 5% Cocaine was insufflated and direct laryngoscopy carried out (by G .C.). A Wisconsin type modified Guedel blade was used with its ordinary illumination supplemented by a standard Jackson laryngoscope light carrier and bulb. The larynx was put through various calisthenics such as high, low and medium pitch sounds, laughter, and short phonation alternating with inspiration. A telescopic camera filmed the sequence at 24 and 64 frames per second using Kodak XXX high speed film. This was then "hot processed." The whole focus of our attention was directed at the posterior rima glottidis and the arytenoid cartilages. The vocal folds were ignored.

The resulting film (Figs 1 & 2) demonstrates an obvious forty-

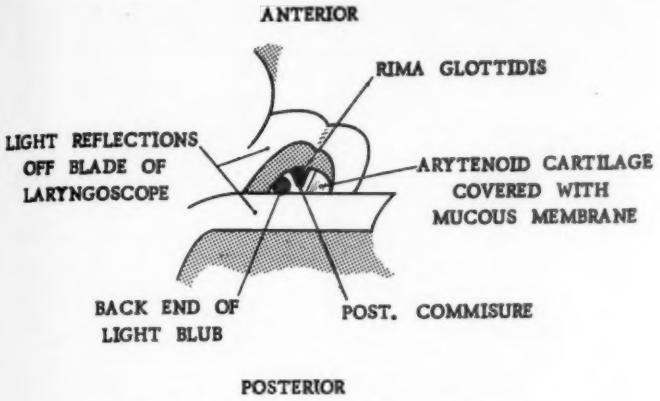

FIG. 1. Schema of structures seen in film strip for orientation.

five degree rotary motion of each arytenoid—making a total ninety degree opening at the posterior commisure. Furthermore, the projected film shows this to be combined with a lateral gliding motion. In fact, the two motions are inseparable. Apparently the resultant of the two components is an arc, the axis of which is posterior and medial to the arytenoids.

It is to be emphasized that our different findings are the result of interpretation and area of camera focus. The use of topical anesthesia did not alter the motion itself since: 1) no musculature could possibly be affected; and 2) close scrutiny of Moore and von Leden's film reveals evidence of the same rotary motion.

Note: Appreciation and acknowledgment of complete dependence for photographic artistry is hereby expressed to Mr. Frank W. Schmidt for this study.

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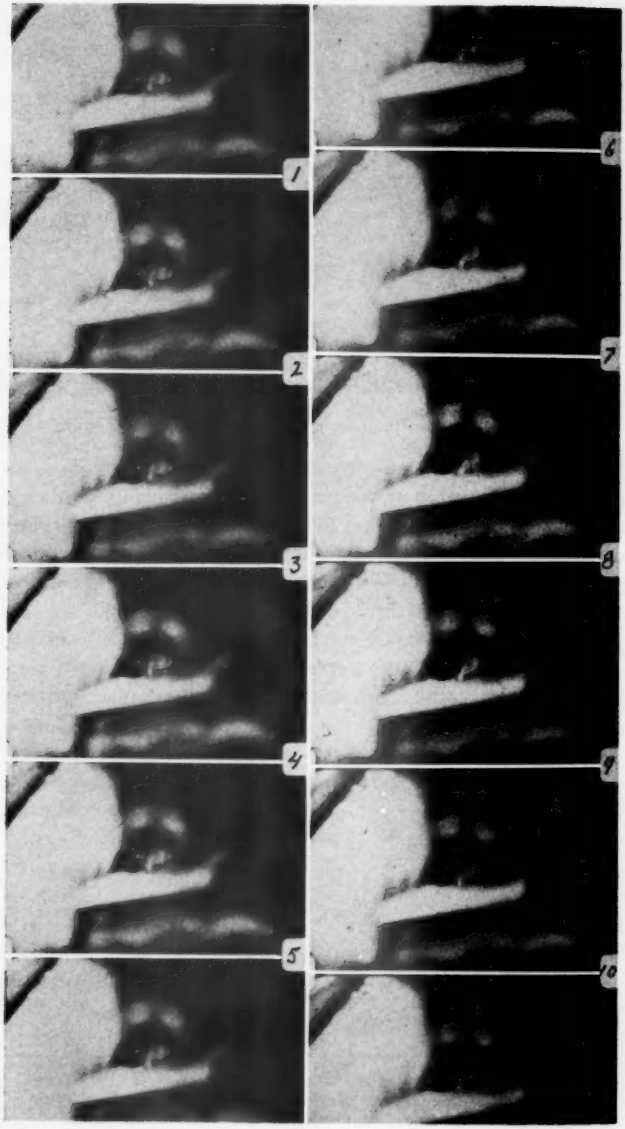
FIG. 2. Film strip of arytenoid cartilages photographed at 24 frames per second showing the opening of the rima glottidis and its almost complete closure. Frame 3 shows complete closure, Frame 6 partial opening and Frame 8 full opening.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A SIMPLE TISSUE CULTURE CHAMBER*

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The need for a container in which mammalian cells can be grown and studied under the microscope has long been recognized. Carrell (1) was one of the first tissue culture workers to attempt to solve this problem and devised the flask which today bears his name. Many workers have at one time or another made use of the hanging drop technique in an attempt to solve the problem of making cultures of cells more available for microscopic study at high magnifications. Such cultures were, however, short lived. Porter (6) adapted the roller tube in an effort to obtain long term cultures and in this he succeeded. However, the flask designed by Porter did not permit the use of high magnifications nor of phase optics. A major step towards solving the problem, made more pressing by the greater availability of high quality phase optics, was taken by Rose (8). The Rose chamber permitted the study of cells growing *in vitro* by means of high magnification phase optics and led to the rapid advancement of time-lapse techniques for the study of growing cells. More recently there have been other chambers devised, which made use of transparent plastic materials in addition to metal and glass. For example such chambers have been described by Christiansen et al (2), by Pulvertaft et al (7), by Toy et al (15), and more recently by Sharp (9) and Cruickshank et al (3). All of these chambers appeared to have some problems associated with them, either difficulty of assembly or of sterilization and maintenance of sterility. A chamber specifically designed to overcome these difficulties was described by Sykes and Moore (10); it was a modification of the chamber that they had made and been using, but was thinner to ensure that all types of oil-immersion phase objectives could be used. Recent changes in the construction of their original chamber have shown that this, more simply constructed, chamber can be used satisfactorily with most oil-immersion phase objectives. Because of the simplicity of construction, ease of assembly, sterilization and handling of this chamber, it

was felt that other tissue culture workers might be interested in the construction and methods of use of this chamber.

Construction

The chamber consists of five parts, two of metal, two of glass and one of silicone rubber. The metal parts are turned from standard stock of stainless steel or brass; when the latter is used, the completed parts are heavily chrome plated to prevent tarnishing and binding of the treads following repeated sterilizations. Aluminum was used in some early experimental chambers but proved unsuitable. If satisfactory operation of the chamber is to be obtained it is important that the dimensions shown in Figure 1 be closely maintained. The metal parts of the chamber are made interchangeable so that no matching of tops with bases is required. No alignment of parts is necessary for the introduction of the needles required in the filling operations. Price considera-

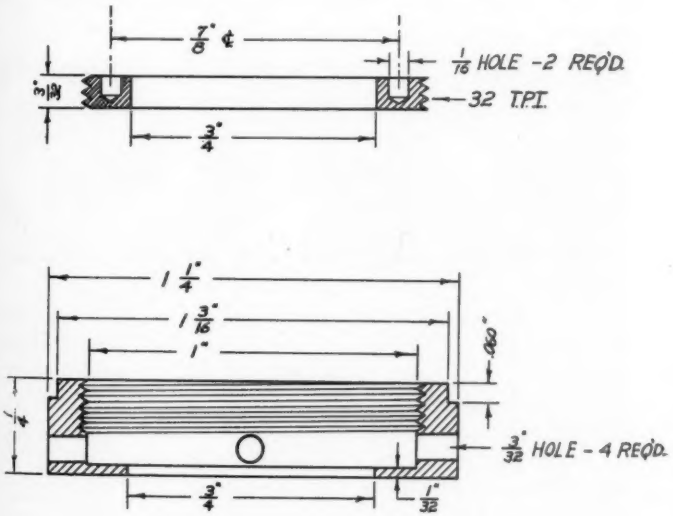


FIG. 1. Mechanical drawing showing dimensions of culture chamber.

ations for a number of chambers make brass the metal of choice for their construction.

One of the metal parts, referred to as the base, has the circumference pierced with four diametrically opposed holes or ports which are used in the filling and emptying operations. The thin metal ring, referred to as the top, screws into the base and is provided with two small blind holes into which fit the pins of a simple annular wrench (Fig. 2a). This wrench is used for tightening the chamber top after assembly. A small aluminum block with a stop pin, which fits into one of the ports in the chamber base may be used to prevent rotation of the chamber during tightening operation (Fig. 2b).

The rubber separating ring which provides a gas and liquid-proof seal between the two cover glasses which form the faces of the chamber is a standard, red silicone rubber "O" ring (American Packing and Gasket Co., Style DC-250, Size 6277–15) of outside diameter 25 mm. This "O" ring has been found to be completely non-toxic to the many cell lines maintained in our

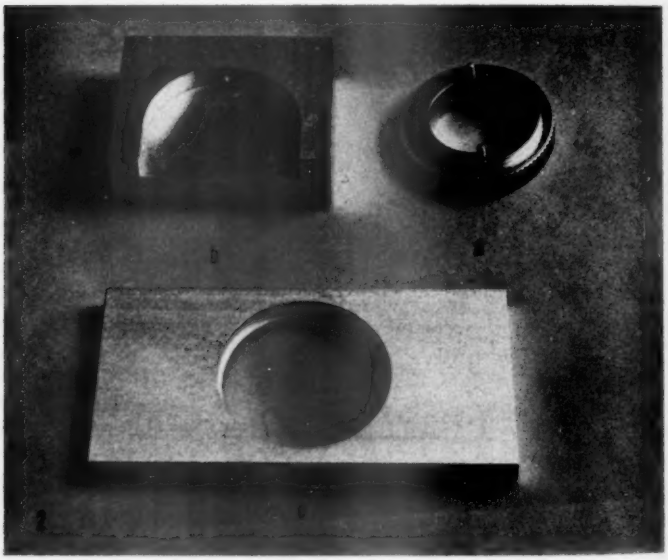


FIG. 2. Accessories for use with culture chamber.

laboratory. Other types of silicone rubber and various formulations of gum rubber have been tested but found to be unsatisfactory. The "O" rings may be repeatedly used until inspection shows them to be perforated to such an extent as not to provide a good bacteria-proof seal. The remaining two parts are the coverslips, which form the faces of the chamber, being 25 mm diameter circular cover glasses of No. 1 or No. 2 thickness. For most purposes the No. 2 thickness has proved suitable and has the advantage of being stronger. The capacity of the chamber is 0.7 ml.

Method of Use

Because of the materials from which the chamber is constructed, sterilization may be accomplished by autoclaving or, if desired, by flaming the individual parts after dipping in alcohol. Autoclaving is the method of choice. Depending upon the technique to be used for loading, the chamber is autoclaved either completely disassembled or assembled except for the screwing into place of the top section. It has been found convenient to sterilize the chambers in petri dishes, using 60 mm dishes for single completely disassembled chambers and 100 mm dishes for five assembled chambers.

The chamber is assembled by placing the base, threaded side uppermost, on a flat surface, dropping into place an alcohol cleaned cover glass followed by an "O" ring and another cover glass. The top is then placed in position and screwed tight. Overtightening of the top will result in rupturing of the cover glasses from entrapped air while insufficient tightening will allow leakage of nutrient and subsequent microbial contamination. Final tightening of the top should be left until the actual filling of the chamber is about to be done. The use of single disassembled chambers has been found useful where it is desired to use plasma clot or Earle's cellophane for the fixation of tissue fragments to the glass. When using Earle's cellophane the following procedure has been found to be satisfactory. Circles of the cellophane, 25 mm in diameter, are soaked overnight in glass distilled water before sterilization by boiling for 30 minutes in fresh double distilled water. The circles of cellophane are then transferred to a 60 mm petri dish containing some of the medium to be used for the tissues under study. Small fragments of the tissue are placed

on the first cover glass, covered with a cellophane circle and the chamber then assembled in the usual way.

The assembled chambers are easily filled with nutrient or cell suspension. A sterile No. 22 or No. 24 hypodermic needle is pushed through one of the ports and by gentle pressure and repeated semi-rotation drilled through the silicone rubber ring. This needle serves as the air vent. With this needle in place the final tightening of the top can be made, one quarter to one half turn is all that should be necessary if the chamber has been properly assembled. A hypodermic syringe of suitable capacity filled with the nutrient or suspension and fitted with a No. 22 or No. 24 needle which is similarly introduced through a port dimetrically opposite the vent needle. The fluid is slowly introduced and the entrapped air removed by suitable positioning of the chamber. Just before the filling operation is completed the chamber is given a sharp tap by striking the edge of the chamber against the bench, this dislodges the small air bubble which clings near the vent needle and permits its discharge. The vent needle is first withdrawn, then the needle on the syringe used in the filling operation. Medium changes are made in a similar fashion, repeated changes of nutrient are possible over many days without fear of microbial contamination. It has not been found necessary when changing the nutrient to sterilize the outside of the "O" ring before insertion of the needles. Using the technique described it has been possible to observe the same cultures continuously over periods of 30 to 60 days.

During the first 24 hours of incubation the chamber is kept base down to permit adherence of the cells to the glass face which will be viewed during microscopy. At the end of this period the medium is changed to remove as much of the cell debris as possible and the chamber re-incubated base side up. This permits any debris not flushed out to fall away from the glass surface to which the cells are adherent and gives a cleaner preparation for photomicrography. For microscopic examination, the chamber is placed in a simple holder (Fig. 2c). This holder will fit the slide clips of any microscope. For ease in handling and ready identification of each culture, the chambers are incubated in the small shelved containers shown in Figure 3. These containers are fabricated from perforated aluminum sheet. The shelves are made from Plexiglass or other suitable plastic in which holes are drilled

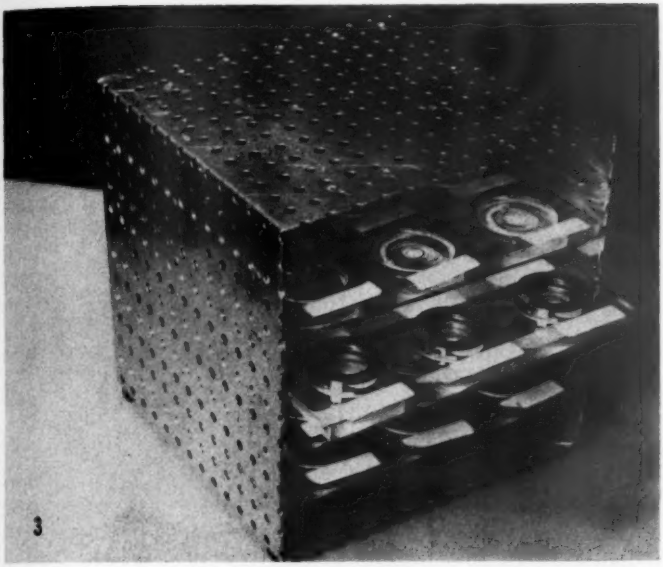


FIG. 3. Container used for incubating chambers.

slightly greater in diameter than the chamber and ⅛ inch in depth. Finding specific cultures is simplified by the use of self-adhesive labels. A complete medium change is obtained with 2 ml of nutrient if the syringe is "pulsed" three or four times during the filling operation. It has been found advantageous to use medium adjusted to a pH of 7.0 to 7.2.

The chamber has been found equally suitable for time lapse studies (5), perfusion experiments and routine microscopy. The chamber has permitted detailed microscopic studies of cells derived from bovine ocular squamous cell carcinoma and its so-called benign precursor lesions (Fig. 4) (11, 12, 13). It has also proved useful in studies on human leukemia (Fig. 5) (4, 14). It is especially adapted for use in virus studies where it is necessary to critically study cellular changes following virus infection (Figs. 6, 7). Cell cultures may be fixed and stained in situ using a similar technique to that employed in medium changing or, preferably, the chamber can be opened and the cover-slip stained in the usual manner.

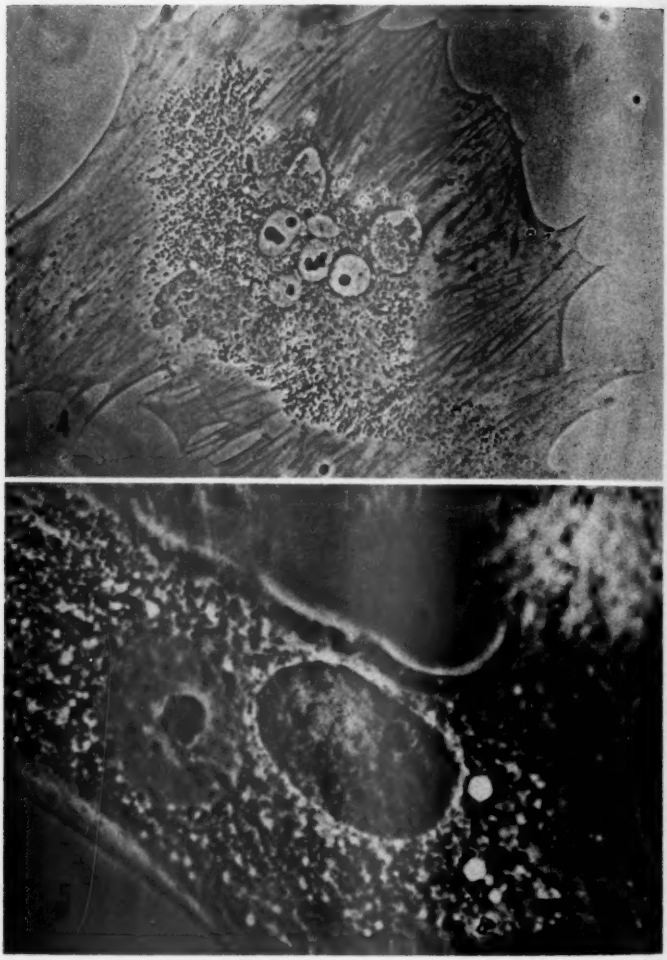
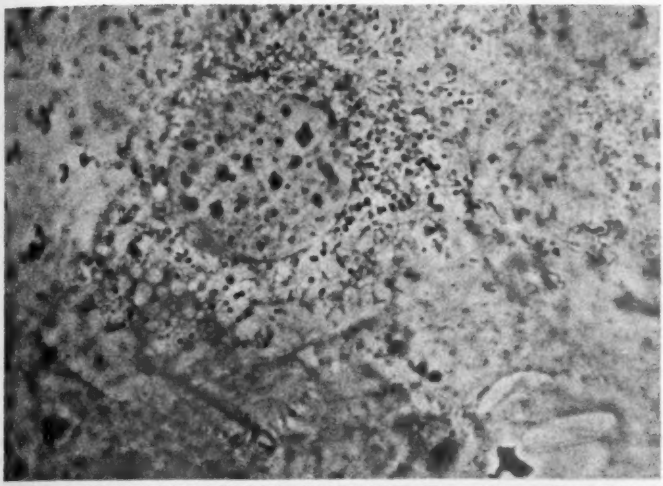


FIG. 4. "Cancer-eye" transitional lesion Plaque—Papilloma, Passage 37: 11 day chamber culture. Cell showing multiple nuclei and cytoplasmic inclusions. x530.

FIG. 5. Lymph node from case of acute myeloblastic leukemia passage 6: 17 day chamber culture. Portion of a cell showing large juxta-nuclear inclusion. x2120.



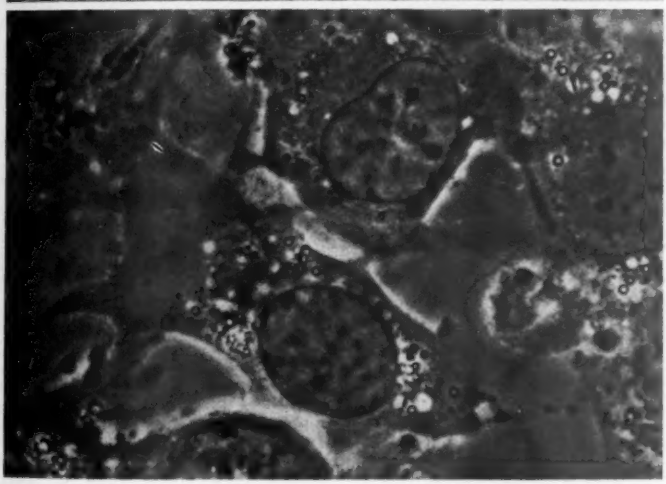


FIG. 6. Stable C_3H minus mouse embryo epithelial cell culture, Passage 65: 13 day chamber culture, control for Fig. 7. x2120.

FIG. 7. Stable C_3H minus mouse embryo epithelial cell culture, Passage 65: 13 day chamber culture after inoculation with S-E polyoma virus. Nuclei show well developed margination of chromatin. x2120.

Summary

A simple and improved tissue culture chamber is described. The chamber consists of 5 parts, 3 of which are standard items readily obtainable. The metal parts of the chamber are easily fabricated from standard stock of stainless steel or brass. The parts of the chamber which are in contact with cells and nutrient are non-toxic. The chamber permits the examination of living cells at high magnification using phase optics. Cell cultures can be maintained and studied over long periods without fear of bacterial or fungal contamination. The chamber is easily assembled and sterilized. The chamber has proved of great value in current studies of material obtained from cases of human leukemia, mouse and chicken leukemia, mouse mammary carcinoma and bovine ocular squamous cell carcinoma and its benign precursor lesions. It has been found especially useful in studies of the action of viruses on various types of cells.

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PARATHYROID ACTIVITY IN NEPHRECTOMIZED RATS[1]

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Past studies concerning parathyroid activity in nephrectomized animals have been presented with two major emphases. One group of papers sought to emphasize that parathyroid hormone was able to produce an effect in the nephrectomized animal, thereby indicating its locus of action in bone (Stoerk, 1943; Stewart and Bowen, 1951; Talmage *et al*, 1953, and Grollman, 1954). The results of these studies demonstrated that parathyroidectomy after nephrectomy, in all species studied, was followed by a drop in serum calcium levels which could be restored toward normal by administration of parathyroid extract; and also, that elevated calcium levels could be produced in the nephrectomized animal by hormone administration. Most of these studies appeared during the controversy concerning the renal and osseous actions of the hormone.

A second emphasis of past work has been concerned with the study of the cytological changes occurring in the gland after nephrectomy. It has been known for some time that, following nephrectomy, the parathyroid gland undergoes a major hyperplasia, and an apparent hyperactivity. Cytological study of these glands has been thwarted by the fact that evidence for secretion, or a change in secretory activity, has been most difficult to establish. This, despite the fact that physiological changes which can be noted in some species within minutes after removal of the parathyroids (Talmage and Kraintz, 1954), indicates that the glands must be continuously secreting their products into the circulation. For this reason the gland in the nephrectomized animal, which does show definite cytological changes within a few days, has received considerable attention (Baker, 1945; Weymouth, 1957; Lever, 1958; Davis, 1959).

A recent emphasis has been found in the reports from this laboratory in studies utilizing the technique of peritoneal lavage (Talmage *et al*., 1957 and 1959). These studies demonstrated that, in both control and nephrectomized rats, bone was able to

continuously supply calcium to extra-osseous areas in both parathyroid-intact and parathyroidectomized rats. However, the amounts of calcium removed and the sites of removal from bone were markedly affected by the presence or absence of the parathyroid glands. Without the activity of the hormone of these glands, the calcium content of extra-osseous spaces, which appeared to be determined by a physico-chemical equilibrium between bone and the fluid compartments of the body, remained significantly below normal levels. A continuous supply of hormone must, therefore, be required to raise the calcium levels of the fluids above this basic level.

Parathyroid activity in the nephrectomized animal is considered by us to provide an excellent example of the ability of these glands to maintain plasma calcium levels even in a stressed condition. The report to follow is a description of the physiological changes which occur in calcium and phosphate metabolism as the result of the increased activity of the parathyroids in this aggravated situation; with the hope that it will help to explain the normal function of the hormone of the parathyroids.

Materials and Methods

Approximately 150 male Holtzman rats weighing between 225 and 250 grams were used in this study. They were maintained on a calcium- and phosphate-free diet for 24 hours prior to experimental use. Nephrectomies were performed through a midventral incision, and the parathyroids were removed individually. All surgical techniques were carried out under ether anesthesia. At sacrifice, the animals were bled by heart puncture, and the serum analyzed for calcium by flame photometry after precipitation with ammonium oxalate and redissolution in perchloric acid and isopropyl alcohol. Phosphate determinates were done after the method of Allport and Keyser (1957). A portion of the plasma was first subjected to 30 minutes of high-speed centrifugation at 14,000 times gravity. This was then analyzed as above.

At the termination of each experiment the femurs were removed and prepared for histological examination according to the method of Toft and Talmage (1960). The parathyroid glands were examined after preparation by histological and histochemical methods. These methods included Zenker's fixation followed by paraffin embedding and tetrachrome staining, post-osmified golgi preparations, and Elftman's Osmichrome fixation followed by staining with Sudan Black B. Observations were correlated with electron microscope findings (examinations made with an RCA-EMT microscope) for a more comprehensive knowledge of the tissue. Hyperactive parathyroids were examined for changes in morphology and lipid content associated with increased secretory activity. Sections of parathyroid glands were scanned with a binocular microscope at 430X magnification and mitotic figures counted in

successive fields as limited by an ocular grid which represented an area of 0.26 mm^2. Osteoclasts were counted using the same ocular grid and magnification.

The Parathyroid Gland in the Nephrectomized Animal

Previous histologists have described marked hypertrophy and hyperplasia of parathyroid tissue after bilateral nephrectomy. Baker (1945) reported a 32% enlargement of the gland, many hypertrophied cells, and a 45-fold increase in the mitotic rate. Nephrectomy obviously provides a stimulus which radically augments parathyroid secretory activity. This stimulus steadily increases, imposing an increasing demand for hormone output as long as the animal lives. The cytological description below is presented as a demonstration of the increased secretory activity of the glands following nephrectomy and is given as a background for the explanation of the physiological events produced by nephrectomy which follows later in this report. These descriptions are not intended to be comprehensive. The reader is referred to Davis (1959) for more detail.

The Normal Parathyroid Gland: Parenchymal cells of the normal gland present considerable heterogeneity of appearance. However, a generalized description is possible. The cells tend to be polarized with basal nuclei and apical golgi. Mitochondria are relatively unoriented. The cytoplasm is moderately acidophilic without localized regions of cytoplasmic basophilia or readily distinguishable secretory antecedents. A few cells are smaller, more acidophilic, and have denser nuclei. They are considered to be identical to the osmiophilic cells described from post-osmified sections. While others (Rosof, 1934; and DeRobertis, 1941) have interpreted these cells as being in terminal stages of a secretory cycle, they are interpreted here as pycnotic or senescent elements. The abnormally vacuolated endoplasmic reticulum and swollen mitochondria displayed by these cells in electron micrographs further suggest cellular derangement, not actively metabolizing cells. If these are senescent elements, the heterogeneity of parathyroid cells is less apparent, weakening the concept of a cyclic sequence of morphological appearances during synthesis and secretion of hormone material.

Lipid droplets are sparse and randomly distributed in light microscope sections. Colloid-like granules have been described

previously from electron micrographs of monkey parathyroids (Trier, 1958). Such granules can be found in the rat (Davis, 1959) and present a moderately electron-dense homogeneous appearance similar to intracellular droplets of thyroid tissue (Dempsey and Peterson, 1955). Whether these are concerned in hormone secretion has not as yet been conclusively demonstrated.

Hyperactive Parathyroid Cells: Several features of this condition have been elucidated by light and electron microscope methods. Within 48 hours after nephrectomy, hypertrophy and hyperplasia are extensive, cytoplasmic basophilia has markedly increased and the golgi apparatus has hypertrophied. Practically all cell components are involved in the hypertrophy. Plasma membranes surrounding the enlarged cells display more irregular foldings interdigitating with those of neighboring cells. Mitochondria appear to be slightly larger and more numerous and tend to be more basally oriented. Also, RNP particles are more abundant and more membranes of the endoplasmic reticulum are present, principally as elongated or small vesicular cisternae, rather than as distended sacs previous described (Lever, 1958).

The appearance of collections of golgi membranes is comparable to that found in normal cells, except that there are numerous secretory droplets associated with them. These droplets are of similar density to those of normal cells, but are somewhat larger in size. Although most prevalent in the golgi zone, they are present throughout the cytoplasm. As anticipated from light microscope findings, lipid droplets are most often found in the basal cytoplasm, frequently associated with mitchondria and masses of the electron-dense granular material.

Osteoclast Changes in the Femur of the Rat Following Nephrectomy

Toft and Talmage (1960) have recently demonstrated that a quantitative determination of osteoclast changes in a specific area of the femur of the rat could be used as an index of parathyroid secretory activity. For these experiments, the osteoclast count was taken at indicated intervals after nephrectomy as a further illustration of the intense activity of the parathyroids produced by nephrectomy. These data are summarized in Table I. An increased count can be noted as early as six hours following

TABLE 1

Effects of nephrectomy on osteoclast concentration and on plasma calcium and phosphate values

Group	No. of rats	O/F[1]	Plasma values	
			mg Ca/100 ml	mg P/100 ml
Normal	32	3.55 ± .07	11.0 ± .2	7.3 ± .2
NEPX 6 hrs.	6	4.67 ± .13	12.4 ± .4	12.5 ± .4
NEPX 12 hrs.	7	5.36 ± .15	10.6 ± .1	13.5 ± .2
NEPX 18 hrs.	6	5.42 ± .47	11.8 ± .4	13.4(2)
NEPX 24 hrs.	16	6.95 ± .32	11.6 ± .4	13.7 ± .5
NEPX 32 hrs.	6	5.93 ± .74	11.1 ± .4	17.3 ± .6
NEPX 48 hrs.	20	5.91 ± .14	12.3 ± .1	16.6 ± .4
NEPX-PTX 24 hrs.	5	3.53 ± .27	6.7 ± .3	15.8 ± 1.0
NEPX-PTX 32 hrs.	11	3.69 ± .45	7.4 ± .5	15.1 ± .9

[1] Osteoclasts per field (field size = 0.26 mm²).

[2] $\text{S.E.} = \sqrt{\frac{\frac{\Sigma X^2 - M}{n}}{n-1}}$

removal of the kidneys. A maximum but more variable stimulation is seen 24 hours post-operative. After this period, the values stabilize as indicated by the standard deviation, but tend to drop slightly. Since these changes were not produced in the nephrectomized animal, if parathyroidectomized, there can be little doubt that they indicate marked increases in the secretory activity of the parathyroids.

While experiments such as these do not necessarily indicate that the hormone works through the osteoclasts in achieving its results on bone, they do demonstrate that following nephrectomy there is a drastic increase in the rate of dissolution of bone. The significance of these data will be discussed later, when they will be used as evidence that an increased amount of calcium is returned to the plasma by the overactive parathyroid gland produced by nephrectomy.

Calcium and Phosphate Plasma Values after Nephrectomy

The effects of renal failure or nephrectomy on plasma calcium or phosphate values are well established. There is an immediate rise in blood phosphate which reaches, in the rat, a value of well over 10 mg P/100 ml. of plasma in six hours after kidney removal. From that time until the death of the animal there is a

progressive increase in this value. It is not unusual to obtain values of 20–30 mg P/100 ml. for plasma phosphate by the third or fourth day. More often a plateau, in the range of 18 mg P/100 ml. is reached usually by 48 hours, and is maintained until the death of the animal. Calcium values, on the other hand, remain relatively constant in the normal range throughout. These data are summarized in Table I. The activity of the parathyroids following nephrectomy is shown by the fact that parathyroidectomy causes a marked drop in plasma calcium with little or no effect on the phosphate values.

Increased secretory activity of the parathyroids has been indicated earlier in this report both by changes in the gland itself, and the changes in the osteoclast count which has been established as a secretory index. Recently, we have given further evidence indicating that a decrease in circulating calcium levels is the stimulus for increased parathyroid secretory activity (Talmage and Toft, 1960). The following data are presented to show that despite the apparent consistency of the calcium values there is an effective reduction in circulating ionized calcium, sufficient to serve as the continuous stimulus for increased parathyroid secretion.

The works of Strates *et al.* (1957) and those reported earlier from this laboratory (Talmage *et al.*, 1960) confirm studies done in 1929 (Shear *et al.*) showing that plasma is not normally saturated with Ca and HPO_4-ions. In our studies, saturation of rat plasma was reached when the product of these two ions is between 50 and 60 (plasma ionized calcium X plasma phosphorus values). If one were to assume that one half of the plasma calcium is ionized, it is easy to calculate that the product of these two ions in the plasma of nephrectomized rats surpasses this by a considerable sum. To illustrate that calcium and phosphate must exist in some other form, presumably as a colloidal complex, in the nephrectomized condition, plasma taken from nephrectomized rats was centrifuged both at normal speeds and at 14,000 X gravity. The results are shown in Table II. The higher speed of centrifugation removed from serum sufficient calcium and phosphate to reduce the product of these two ions to within the 50 to 60 range. The remaining calcium value is therefore lower than normal, and serves as a stimulus for the increased parathyroid activity known to occur following nephrectomy.

TABLE 2

Effect of high-speed centrifugation on plasma calcium and phosphate values[1]

Group	Ca BHSC[2]	Ca AHSC[3]	PO_4 BHSC	PO_4 AHSC
Control	11.61 ± .15	11.67 ± .09	6.16 ± .29	6.42 ± .25
NEPX 36 hrs.	11.07 ± .37	8.03 ± .36	17.29 ± .60	14.88 ± .64
NEPX 48 hrs. + 10 mg Ca/4 hr.	11.16 ± .12	11.06 ± .10	8.89 ± .54	9.00 ± .54

NOTE. NEPX = Nephrectomized.
[1] Modified from Wimer, 1959.
[2] Before high-speed centrifugation.
[3] After high-speed centrifugation.

The Effect of Calcium Administration on Nephrectomy Changes

The earlier sections of this report have demonstrated the increased activity of the parathyroids following nephrectomy. They have also suggested that this increased activity was due to a decrease in circulating ionic calcium levels. If this be true, then one would expect marked reversal of these events following the administration of calcium to the nephrectomized animals. The following experiments were done to illustrate this point. Calcium was given by stomach tube in amounts indicated, at four hour intervals. The changes in the sequence of events normally seen following nephrectomy are summarized in Table III. The largest dose of calcium used, 15 mg/4 hours, returned the osteoclast count to normal, maintained slightly elevated plasma calcium values, while dropping the phosphate back into the normal range. High speed centrifugation studies as illustrated in Table II demonstrated that no colloidal calcium phosphate existed in the serum, and that the ion product was well within the normal range. The only factor that was not completely returned to normal was the mitotic count in the parathyroid tissue which still remained elevated.

The Relationship of Circulating Calcium and Phosphate to Bone

In order to interpret these phenomena which occur in the nephrectomized rat, it is necessary to review briefly the relationship of the calcium and phosphate of the circulating fluids to the solid phases of bone. While it has been shown that plasma and extracellular fluids are not saturated with these two ions, *per se*,

TABLE 3

Effects of calcium administration on mitotic activity of the parathyroid gland and on osteoclast concentrations in nephrectomized rats

Group	Plasma values				
	O/F[1]	mg Ca/100 ml	mg P/100 ml	M/F[2]	X normal
Normal	3.55 ± .07(32)[3]	11.0 ± .2(38)	7.3 ± .2(38)	0.141 ± .21(14)	1
NEPX 24 hrs.	6.95 ± .32(16)	11.6 ± .4(16)	13.7 ± .5(16)	0.163 ± .03(5)	..
NEPX 48 hrs.	6.91 ± .14(20)	12.3 ± .1(20)	16.6 ± .4(20)	4.11 ± .19(13)	29
NEPX 48 hrs. 5 mg. Ca/4 hrs.	6.33 ± .36(7)	12.9 ± .2(7)	9.8 ± .3(7)		
NEPX 48 hrs. 10 mg. Ca/4 hrs.	4.76 ± .24(10)	11.2 ± .1(10)	8.9 ± .4(10)	1.27 ± .20(7)	9
NEPX 48 hrs. 15 mg. Ca/4 hrs.	3.69 ± .21(8)	13.2 ± .5(8)	7.6 ± .2(8)		
NEPX 72 hrs.	4.75 ± .69(5)	9.6 ± .8(5)	24.4 ± 1.3(5)	4.04(2)	29
NEPX 96 hrs.				4.29(1)	29

[1] Osteoclasts per field. (field size = 0.26 mm²).
[2] Number of mitoses per field (field size = 0.26 mm²).
[3] Number of animals.

the same fluid passing through bone is supersaturated in respect to the bone crystals. While in the vicinity of bone, therefore, calcium and phosphate would be precipitated from fluid to the solid phases. One would assume, further, that, should the fluid concentration drop below this equilibrium point, the solid phases would supply both of these ions to the circulating fluids. That this does in effect occur has been demonstrated by the works of Hastings and Huggins (1933), Copp (1957), and by Talmage *et al* (1957). This basic physico-chemical equilibrium is only approached in the parathyroidectomized condition or by experimental means. In the normal condition, circulating values are well above the equilibrated level, maintained there by the continuous action of the parathyroid hormone on bone, feeding back calcium and phosphate into the body fluids.

It is obvious, therefore, that on the one hand, the basic physico-chemical system is continuously attempting to equilibrate, while the parathyroid hormone, by an equally continuous action, is returning calcium and phosphate to the body fluids against this equilibrium gradient. This situation provides a constant precipitation of bone salts even with a changing supply of exogenous calcium, and also makes it necessary for the bone to supply calcium continuously, thereby giving the impetus for bone remodel-

ling. It follows also that the rate of precipitation depends upon the difference in the equilibration level and the sum of the Ca^{++} and $HPO_4^{=}$ ions in body fluids. Therefore, if the plasma concentration of either of these two ions is increased, there is a temporary increase in the rate of precipitation in bone. However, since in all mammals (and most other vertebrates, as well) the calcium level of plasma is remarkable constant and controlled, the environment for the proper growth and remodelling of bone is, by the same token, maintained within its optimum range at all times.

Parathyroid, Plasma, Bone Relationships in the Nephrectomized Rat

The data presented here demonstrate that following nephrectomy there is a marked increase in the activity of the parathyroid tissue, a major speeding up of the resorptive processes in bone, and a rising concentration of phosphates in the body fluids, all of which are accompanied by a relatively constant calcium concentration of the plasma. They have also demonstrated that the only factor needed to reverse these changes is the administration of calcium to the animal.

To explain these phenomena one must begin with the phosphate. Phosphate is excreted in relatively large amounts by the kidney. When the kidneys are removed, circulating phosphate values rise. This immediately increases the gradient of precipitation of calcium and phosphate from fluid to bone by the processes explained above. Without an equivalent source of extra-osseous calcium to match the phosphate, plasma calcium levels tend to fall. This immediately produces an increased activity of the parathyroid gland causing an increased reabsorption of bone, and increases the amount of calcium returned to the circulating fluid in an attempt to maintain the normal calcium levels. Unfortunately, phosphate as well as calcium is released from bone, and additional phosphate from other endogenous sources continues to be added to the circulating fluids. Intense activity of the parathyroids ensues, and as long as the output of the gland can match the need, calcium values for plasma appear to remain constant, though obviously somewhat depressed.

The stress on the gland is such that only by this means can hypertrophy and hyperplasia be demonstrated in a short period

of time, in this case within 48 hours. Calcium administration gives an exogenous supply of calcium to match the phosphate thus precipitating these ions in bone without requiring the parathyroids to furnish the additional calcium. Changes in gland, bone, and plasma phosphate are therefore negated and, in these regards, the animal remains in a normal physiological condition.

Conclusions

The phenomena discussed in this paper represent an abnormal condition resulting from the extreme stress put on the animal by failure of excess phosphate derived from metabolic sources to be excreted. This nephrectomized condition does, however, give an excellent demonstration of the mechanism of action of the parathyroid hormone in the body in respect to its action on bone. In the final analysis, the function of the hormone is to maintain a constant level of circulating calcium in plasma and extracellular fluids. By this function, it maintains the most optimal calcium level for the many physiological processes into which calcium enters. One of the most important of these is the maintenance of an environment which supplies a constant amount of the necessary salts for bone growth. At the same time, the action of the hormone requires the return of these salts to the circulating fluids from bone, thereby providing the necessary impetus for proper bone remodelling.

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BIOASSAY OF ANTERIOR PITUITARY HORMONES OF THE RAT AFTER SEVERE STRESS

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Timmer and Finerty (1956) demonstrated a marked decrease in weight of the pituitary gland of albino rats twelve hours after scalding. Both wet and dry weights of the pituitary expressed in either absolute or relative terms showed a marked decrease. Concurrent with loss in weight, the number of cells per microscopic field was increased and this was interpreted as representing a decrease in cell size. An obvious suggestion to explain the marked loss in weight would be rapid hormonal discharge. This report presents the results of further investigation of this suggestion by applying bioassay methods to measure hormonal content of the anterior pituitary of albino rats after scalding.

Materials and Methods

Male Holtzman rats weighing 114–118 gms. were scalded in 70° C water for five seconds while under nembutal anesthesia. They were sacrificed at periods of 1, 3, 12 and 24 hours after stress. The anterior pituitaries were removed, weighed on a Roller-Smith torsion balance, (crushed) between clean microscopic slides and dried in a desiccator over calcium chloride. The dry powder obtained from the anterior pituitary glands of the several groups of rats was used in the bioassay of gonadotrophin, thyrotrophin and growth hormone. These three hormones were assayed by using twenty-four-day-old hypophysectomized female rats. The assay animals were given subcutaneous injections at dose levels of 1.0 mgs. and 2.0 mgs. of dry pituitary powder over a four and one-half day period with autopsy on the fifth day.

Measurement of body weight and width of the epiphyseal cartilage of the tibia as described by Greenspan *et al* (1949) were used as criteria for growth hormone. Weight of the thyroid gland and height of the follicular epithelium were used as criteria of thyrotrophin activity. Weight of ovaries and uteri were used as an index of total gonadotrophin content.

Bioassay for ACTH utilized hypophysectomized male rats weighing approximately 150 gms. The adrenal ascorbic acid depletion method of Sayers, Sayers and Woodbury (1948) was utilized using two dosages of 0.5 mg. and 1.0 mg. of anterior pituitary tissue extracted by 0.1 N HCl. The anterior pituitary tissue used for ACTH assay was obtained from a pool of ten animals killed at each of the following periods after scalding: immediately, fifteen and thirty minutes, 1, 3, 12 and 24 hours. Results are expressed as percentage depletion of adrenal ascorbic acid and as ACTH units in thousandths de-

termined by comparison to ascorbic acid depletion caused by known amounts of ACTH.

Other methods of stress applied to approximately 150 gms. male rats included (1) Cold, by placing rats in a refrigerator for twenty-four hours at 8° C, (2) Hemorrhage, by withdrawing two ml. of blood from the heart with a syringe and needle, (3) Forced swimming for thirty minutes, (4) Electric shock by maintaining convulsive rigidity for ten seconds using an A.C. current of ten volts and 45 milliamperes applied to the ears. All animals were autopsied twelve hours after stress.

Pituitary tumors were produced by placing 15 mg. tablets of methyl stilbestrol subcutaneously in sixty female rats weighing 60–80 gms. After sixty days, one-half of the animals were autopsied as tumor controls. The remaining animals were scalded as described above and autopsied twelve hours later and the anterior pituitary weighed. The PAS-methyl blue technique of Wilson and Ezrin (1954) was used for a cytological study of these tumors.

Appropriate controls were maintained for all scalded and bioassay groups.

Results

Table I demonstrates a decrease in weight of the anterior pituitary at twelve hours after scalding. This is noted in both the absolute and relative weight with the maximum decrease occurring at twelve hours after scalding. Table II shows the results of the assay of growth hormone in the pituitary powder obtained from the various groups of control and scalded animals. Differences in the response of the epiphyseal cartilage to the injection of the various pituitary powders indicate a significantly lower

TABLE 1

Weight of the anterior pituitary gland of severely burned rats

Group	No. of animals	Body wt./gms.	Ant. pit. wt./gms.	Ant. pit. wt./100 gms. body wt.
Controls	73	118	3.59	3.04
		±.97[1]	±.04	±.03
1 Hour post-stress	72	117	3.55	3.04
		±.62	±.04	±.04
3 Hour post-stress	72	115	3.29[2]	2.86
		±.60	±.04	±.03
12 Hour post-stress	72	116	3.25[2]	2.80[2]
		±.78	±.04	±.03
24 Hour post-stress	74	114	3.39	2.98
		±1.05	±.04	±.04

[1] Standard error.
[2] P value = <.01.

TABLE 2

Body weight changes and width of uncalcified epiphyseal plate of tibia in hypophysectomized assay animals receiving anterior pituitary powder in saline solution

Pituitary donor group	1 Mg. dose				2 Mg. dose			
	No. of assay animals	Body weights[3]	Diff.	Width of epiphyseal plate in μ	No. of assay animals	Body weights[3]	Diff.	Width of epiphyseal plate in μ
None (Saline injected)	7	74–78 ±2.4[1]	4	194 ±6.2	5	51–59 ±1.4	8	251 ±7.1
Control Non-stressed	6	71–81 ±3.8	10	293 ±8.1	6	51–69 ±2.4	18	380 ±6.8
1 Hour post-stress	5	69–82 ±11.4	13	276 ±7.6	6	50–66 ±2.5	16	376 ±9.5
3 Hour post-stress	6	76–88 ±7.9	12	270 ±9.4	6	52–66 ±3.3	14	347[2] ±7.2
12 Hour post-stress	5	73–84 ±3.5	11	254[2] ±7.2	5	48–65 ±6.3	18	358 ±8.3
24 Hour post-stress	5	74–83 ±7.8	9	272 ±10.1	5	49–65 ±2.8	16	365 ±9.8

[1] Standard error.
[2] P value = <.01.
[3] First number indicates original body weight, second number indicates autopsy weight, to which standard error applies, also these body weights apply to same animal groups in Table 3–5.

response to the 1.0 mg. dose of the powder from the twelve hour post stress animals as compared to that obtained with the same dose of pituitary powder from the control animals. The same can be said for the tibial response of the animals given the 2.0 mg. dose of powder from the three-hour post stress group. Body weights are variable between groups with no significant differences noted. Note also that all assay animals receiving the 2.0 mg. dose have a lower original body weight than those receiving 1.0 mg. of pituitary powder. The author has no explanation for this difference in weight except that these assay animals were ordered separately but were supposedly of the same age. The response in terms of the weight of the thyroid, ovaries and uteri is markedly increased over the animals receiving the 1.0 mg. dose. Table III shows there is a significant increase in the height of the thyroid follicular epithelium of the assay animals given the twelve hour post-stress pituitary powder at both the 1.0 and 2.0 mg. dose. No changes were noted in the weight of the thyroid among the various stress groups at either dose level. In Table IV the bioassay

TABLE 3

Assay for thyrotrophic hormone

Pituitary donor group	1 Mg. dose			2 Mg. dose		
	No. of assay animals	Weight of thyroid mg.	Height of epithelium μ	No. of assay animals	Weight of thyroid mg.	Height of epithelium μ
None	7	4.34	3.33	5	6.05	3.98
(Saline injected)		±0.36[1]	±0.41		±0.64	±0.40
Control	6	6.13	8.14	6	8.08	9.15
Non-stressed		±1.24	±0.38		±0.31	±0.36
1 Hour	5	5.59	9.58	6	7.04	9.97
post-stress		±0.28	±0.82		±0.36	±0.57
3 Hour	6	6.16	9.70	6	6.81	9.91
post-stress		±0.59	±0.73		±0.57	±0.83
12 Hour	5	6.35	9.96[2]	5	7.86	10.30[2]
post-stress		±0.58	±0.34		± .39	±0.33
24 Hour	5	6.00	7.00	5	6.99	9.97
post-stress		±0.84	±0.81		±0.41	±0.56

[1] Standard error.
[2] P value = <.01.

TABLE 4

Assay for gonadotrophic hormones

Weight of the uterus and ovary in hypophysectomized assay animals receiving anterior pituitary powder in saline solution

Pituitary donor group	1 Mg. dose			2 Mg. dose		
	No. of assay animals	Uterine weight mg/100 gms.	Ovarian weight mg/100 gms.	No. of assay animals	Uterine weight mg/100 gms.	Ovarian weight mg/100 gms.
None	7	40	14	5	34	13
(Saline injected)		±4.5	±1.7[1]		±.37	±1.2
Control	6	276	88	6	257	197
non-stressed		±26.1	±7.4		±10.5	±22.9
1 Hour	5	255	102	6	262	188
post-stress		±14.3	±9.4		±8.2	±46
3 Hour	6	242	82	6	271	188
post-stress		±20.4	±7.9		±9.4	±7.4
12 Hour	5	232	112	5	259	208
post-stress		±31.6	±13.4		±14.5	±18
24 Hour	5	265	101	5	223	222
post-stress		±23.6	±15.5		±7.5	±26.9

[1] Standard error.

for gonadotrophic hormones shows no significant differences among the various assay groups in the weight of the ovaries or uteri.

Table V shows results of the ACTH assays. The pituitary content of ACTH is expressed as the percentage drop in adrenal ascorbic acid and as ACTH units (Armour ACTH was used as a reference standard). From a normal value of 9.8 milliunits per 0.5 mg. tissue there is an increase in ACTH content of the anterior pituitary in the immediate, fifteen and thirty minutes post stress groups up to 13.5 milliunits. This is followed by a drop in ACTH content at one hour after stress to 6.5 milliunits and it remains at a lowered level throughout the first twenty-four hours. A similar pattern of results is found in animals receiving 1.0 mg. of anterior pituitary tissue, although at this dosage the peak value is seen at 15 minutes after stress.

Table VI shows the weight of the anterior pituitary at 12 hours after using various forms of stress other than scalding. There is

TABLE 5

ACTH assay in hypophysectomized animals injected with anterior pituitary homogenate of stressed animals

	0.5 Mg.			1.0 Mg.		
Donor group	No. of assay animals	Percent drop	Drop in ACTH units in thousandths	No. of assay animals	Percent drop	Drop in ACTH in thousandths
Controls	13	28.8	9.8 ±.19[1]	12	35.8	18.8 ±.21[1]
Immediate	14	31.7	12.8 ±.33	12	38.2	21.6 ±.13
15 Min. P.S.	13	31.4	12.0 ±.29	14	40.7	28.0[2] ±.24
30 Min. P.S.	14	32.2	13.5[2] ±.30	13	39.8	26.0 ±.10
1 Hour P.S.	12	26.0	6.5 ±.24	15	32.6	13.8 ±.20
3 Hours P.S.	13	23.9	4.8 ±.31	10	32.8	14.0 ±.18
12 Hours P.S.	12	22.6	4.2[2] ±.18	10	29.6	9.8[2] ±.28
24 Hours P.S.	12	22.0	3.8[2] ±.20	10	28.8	9.8[2] ±.25

[1] Standard error.
[2] P value = <.01.

TABLE 6

Weight of the anterior pituitary after other types of stress

Group	No. of animals	Body wt.	Ant. pit. wt.	Ant. pit. wt./100 gms. body wt.
Controls	30	148	4.17	2.82 ±.04[1]
Cold	30	155	4.39	2.81 ±.04
Swimming	30	138	4.38	3.12[2] ±.03
Hemorrhage	27	142	4.28	3.01 ±.06
Electric shock	30	137	4.04	2.95 ±.04

[1] Standard error.
[2] P value = <.01.

TABLE 7

Weight of estrogen induced tumors of anterior pituitary of burn stressed rats

Group	No. of animals	1st body wt.	Autopsy body wt.	Ant. pit. wt./100 gms. body wt.
Controls	9	75	386	2.32 ±.06[1]
Stilbestrol	27	71	165	6.01[2] ±0.28
Stilbestrol & stress	25	66	153	6.44[2] ±.07

[1] Standard error.
[2] P value = <.01.

no significant decrease in weight of the gland of any of the stressed groups but an increase in weight of the gland is seen in animals stressed by swimming. Table VII gives the relative weight of the anterior pituitary in animals receiving methyl stilbestrol as compared to control animals. No decrease in weight of the tumorous anterior pituitary glands of the stilbestrol-treated rats was observed after scalding.

Discussion

The results in Table I are comparable to those found in earlier work by Timmer and Finerty (1956) in which the whole pitui-

tary showed the most marked decrease in weight twelve hours after scalding. The results shown in Tables II, III, and IV for bioassay of thyrotrophin, gonadotrophin and growth hormone are perhaps not as informative as might have been obtained with separate assay techniques for the different hormones using several groups of animals, but it was felt that the method used would detect any marked differences in the pituitary content of these hormones. Realizing this and utilizing more than one criterion for each hormone the results indicate a probable increase in thyrotrophin content of the anterior pituitary twelve hours after stress as demonstrated by the increased height of thyroid follicular epithelium in assay animals. The results of the tibia test may indicate a decrease in growth hormone content at three to twelve hours after stress. This decrease in growth hormone at twelve hours after stress is in contrast to the results reported by Knigge (1958) using similar techniques of stress. It should be pointed out that in both these studies other hormones such as ACTH, thyrotrophin and prolactin may interfere with the tibial response. The other criteria used to measure growth hormone, thyrotrophin and gonadotrophin are too insensitive by the assay procedures followed to detect any other changes. The results obtained in this study apply only to anterior pituitary hormone content and give no information as to the rate of synthesis or rate of secretion of these hormones.

The results shown in Table V for ACTH content were obtained by using standard assay procedures described in the literature and the results appear uniform for the two dose levels. There is a definite increase in ACTH content during the first hour with a precipitous drop in content one hour after scalding. The level of pituitary ACTH shows no return toward normal during the tweny-four hour period studied.[b]

The results obtained after scalding the stilbestrol-treated animals (Table VII) give some evidence for growth hormone being involved in the weight reduction of the anterior pituitary. Meyer and Clifton (1956) described these pituitary tumors as containing mostly acidophiles and chromophobes which were correlated with a high prolactin content and a deficiency in other pituitary hormones, particularly growth hormone. Lacour (1950) described the acidophiles of these tumors as staining orange with the Romeis pituitary stain and as being different

from the acidophiles usually associated with growth hormone. The work of Sanders and Rennels (1959) further established these orange acidophiles as being specifically related to prolactin content of the anterior pituitary. Thus, a failure to find a decrease in weight of these pituitary tumors following a scald might be due to a deficiency of the acidophile cell responsible for growth hormone production.

The failure to obtain a similar loss in weight twelve hours after using various forms of stress as indicated in Table VI, may indicate that scalding has a peculiar ability to produce a loss in weight of the anterior pituitary. One must remember that these other forms of stress are less severe and do not lead to the hemoconcentration seen with a scald.

The reduction in weight of the anterior pituitary and concomitant decrease in cell size twelve hours after stress found in this and earlier studies might be accounted for in part by the results noted here. At least the indication of a reduction in growth hormone as reflected by the tibial response and the drop in ACTH content represents a positive loss of substance which is apparently uncompensated twelve hours after stress. In addition, the increase in thyrotrophin content, and the unchanged values for gonadotrophins are indicative of the highly selective effect of the scald. These facts in turn tend to minimize the importance of general factors such as hemoconcentration with the associated changes of water, electrolytes and diffusible cellular proteins in accounting for the reduction in pituitary weight.

Summary

The effects of a severe stress, scalding, were studied in the male albino rat in relation to hormone content of the anterior pituitary. ACTH assay indicated a brief increase in ACTH content followed by a marked and rapid decrease one hour after stress. No return toward a normal level of ACTH content was seen up to twenty-four hours after scalding. Bioassay methods indicated a probable decrease in growth hormone content three to twelve hours after stress. There were no significant changes found in the content of gonadotrophic hormones, whereas increase in thyrotrophin content was demonstrated at twelve hours after stress. Animals in which pituitary tumors were induced by stilbestrol treatment

were also subjected to scalding. In contrast to the normal animals, these failed to show a decrease in the weight of the anterior pituitary gland twelve hours after stress. The effects of various other stressors on anterior pituitary weight were studied and it was found that only scalding led to a marked reduction in the weight of this gland. It is concluded that the decrease in hormone content of the anterior pituitary gland seen at twelve hours after scalding may account in part for the corresponding loss in weight of the gland.

Acknowledgments

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[a] This study was supported by Navy Contract NONR 1598(03).

[b] Since these data were presented in dissertation form a report by Holub, Kitay and Jailer (1959) has appeared giving a similar pattern of change in ACTH content of the anterior pituitary after scalding.

This study was presented for Ph.D. dissertation by the author June, 1959 at the University of Texas Medical Branch, Galveston, Texas, and published in Dissertation Abstracts, L. C. Cord No. Mic 59–4750, May, 1959.

EFFECTS OF TRANSPLANTATION ON THE HISTOGENESIS OF TRANSITIONAL EPITHELIUM

BRUCE E. WALKER and JOSEPH L. PRUD'HOMME

Polyploid nuclei arise by fusion of metaphases during the synchronous division of binucleate cells in the surface cell layer of transitional epithelium. This takes place in the urinary bladders of 16 day old mouse embryos (4). Subsequently, the polyploid surface cells gradually develop the special characteristics that distinguish them from the other cells of the epithelium. These circumstances were interpreted as a necessary sequence of histogenetic events in this tissue (4). If polyploidy is essential to the further differentiation of surface cells, then this is one case in which histogenesis is accomplished through nuclear differentiation, since polyploidy is a genetic alteration of the nucleus.

This hypothesis could be tested experimentally if a method could be found to prevent the development of polyploidy and still permit the observation of subsequent histogenesis. Since polyploid nuclei have not been seen to form anew from the fusion of diploid nuclei in adult transitional epithelium, the adult may be completely dependent on the supply of polyploid nuclei it receives from the embryonic development of this epithelium. To investigate this possibility, areas of transitional epithelium were destroyed in the adult bladder and then followed radioautographically to see if new surface cells could be produced (5). There was no evidence of polyploid nuclei being formed by fusion of diploid nuclei in this experiment, but surviving polyploid surface cells proliferated to replace those destroyed (5). These results support the hypothesis that polyploidy is essential for the production of surface cells, but the experiment was not a critical test of this hypothesis.

The experiment described below is based on the transplantation of embryonic and adult bladders into a subcutaneous location in adult mice. The experiment was designed to discover whether the epithelium from embryos younger than 16 days could develop polyploidy in this new location. If not, then the subsequent behavior of the epithelium might reveal whether

surface cells can arise without polyploidy. Transplantation of bladders from embryos in which polyploidy had already arisen in the epithelium might reveal whether this nuclear event is sufficient to assure the complete differentiation of the surface cells. Pieces of adult bladder were transplanted to serve as controls for evaluating the progress of differentiation in embryonic transplants.

Materials and Methods

Embryos were obtained from heterozygous mice or from C57BL mice mated overnight. The females were checked in the morning for vaginal plugs and fertilization was assumed to have occurred at 2:00 A.M. (2) for purposes of timing embryos. A/Jax, C57BL or heterozygous adult mice were used for hosts, as specified in Table 1. Embryonic bladders were transplanted whole, but adult bladders were cut across and transplanted as separate slices. Small incisions were made in the skin on each side of the midline in the ventral abdominal wall near the hind legs. Subcutaneous pockets were made with a probe lateral to the incisions and several bladders were implanted on each side. Both the donor and host mice were anesthetized with ether for this procedure. The transplants were recovered at intervals of a few days to 5 weeks. After paraffin sectioning of the transplants, the following staining procedures were used: hematoxylin and eosin, periodic acid Schiff, and Feulgen. Control embryos were saved from the litters used for transplanting embryonic bladders and these embryos were used to estimate the developmental age of each litter. To arrive at a morphological rating for these embryos, the same numerical system was applied that had been developed for studying palate embryology (6). Since the morphological rating system does not extend beyond 16 days, the age of older embryos was determined from the morphological criteria published by Grüneberg (1).

Results

A total of 324 embryonic bladders and 306 slices of adult bladder were tranplanted into 105 adult host mice. When recovered before 10 days, the epithelium of the transplants was generally in a state of flux and difficult to categorize. Evidence of epithelial cell migration was often seen at this time. If the embryonic transplants were left for more than 4 weeks, it was usually difficult or impossible to locate them. The optimum time to recover transplants was between 2 and 4 weeks. Often, the bladders implanted in one area fused to each other. Most of the adult bladder slices, and the bladders from embryos of 16 days or older, developed into cysts if the epithelium survived. Cysts did not develop from bladder transplants of 14 and 15 day old embryos except in one case where adult type transitional epithelium was present. Of the

adult bladder transplants recovered, 66 showed healthy epithelium; whereas only 33 embryonic bladders had epithelium reasonably free of necrotic change. In transplanting embryonic bladders, no attempt was made to remove adjacent male genital gland tissue, and often the recovered transplants had large masses of tissue resembling vesicular glands or prostate (Fig. 1). These tissues were not included in Table 1 because they could be clearly distinguished from bladder by the lack of a heavy fibro-muscular wall.

In Table 1, the circumstances and results of the embryonic bladder transplants are given. The list is restricted to recoveries of vesicles with thick, fibro-muscular walls and healthy epithelia. Transplants with necrotic epithelia or with thin connective tissue walls were not included. The estimated age given across the top of the table is based on average values of morphological ratings vs. chronological age for 3 strains of mice, as tabulated elsewhere (3). The 17 day old embryo, for which no morphological rating can be given, was confirmed by Grüneberg's criteria (1).

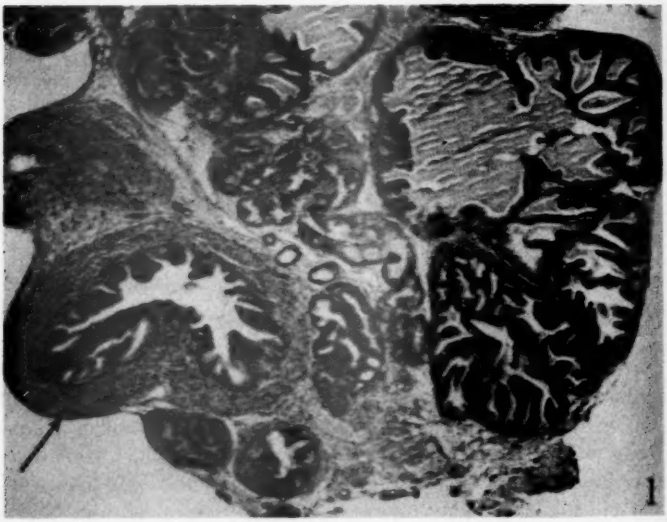

FIG. 1. Low magnification photomicrograph of a 22 day transplant in which male genital tissue predominates. The latter can be easily distinguished from urinary bladder because the bladder has a thick fibro-muscular wall (arrow). Stained with hematoxylin and eosin.

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TABLE 1

Transplants of embryonic bladders into adult mice
(see text, under "Results", for explanation of table)

Estimated age (days post conception)		14	14	14	14	15	15	15	15	15	15	15	16	16	16	16	16	16	17
Morphological rating of donor		7	8	10	10	13	15	15	16	17	19	20	30	30	30	30	30	30	. .
Chronological age of donor	days	15	14	14	14	14	15	15	14	15	16	16	16	16	16	16	16	16	17
	hours	12	9	10	8	11	9	8	8	8	8	10	8	8	8	9	9	10	10
Strain of donor's parents		H	C	C	H	H	H	C	C	H	H	C	H	C	C	H	C	C	H
Strain and sex of host		A♂	C♂	C♂	A♂	A♂	H♀[1]	C♂	C♂	A♂	C♂	C♂	C♂	C♂	C♂	C♂	C♀[2]	C♀[2]	A♂
Duration of transplantation (days)		28	24	24	6	17	14	19	24	28	19	18	26	22	22	26	13	13	18
Type of epithelium recovered																			
Embryonic transitional		.	3	1	.	1	.	1	1	.	1	1	.	.	.	.	.	.	.
Adult transitional		1	.	1	.	.	.	.	1	.	.	.	1	1	3	3	1	1	1
Simple columnar		.	.	1	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Stratified columnar		.	2	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.
Satified squamous, keratinizing		.	.	.	.	.	1	1	.	.	.	1	.	.	.	.	.	.	.
Cartilage		.	.	.	.	.	1	.	.	3	.	.	.	.	.	.	.	.	.

[1] Pregnant, 15 days post conception.
[2] Pregnant, 16 days post conception.

Although chronological age, calculated as the interval between vaginal plug observation and time of transplantation, is given, it shows the usual inaccuracies (6) when compared with the morphological ratings (developmental age). The transplants from embryos 14 to 15 days old are arrayed according to morphological ratings. The 16 day old embryos are arrayed by chronological age since the morphological rating system stops at 30. In the listing of mouse strains, H-heterozygous, C-C57BL, A-A/Jax. Epithelia were classified as embryonic when they resembled the epithelium in 14 or 15 day old embryo bladders (Fig. 2) or when the deviation from normal embryonic type was slight (Fig. 3). Epithelia were classified as adult type transitional epithelium if they had giant surface cells with periodic acid Schiff positive cytoplasm and giant nuclei, resting on two or more layers of small cells (Figs. 4 and 5). Since the relevance of polyploid nuclei in adult transitional epithelium was being tested, the presence of large surface cells without giant nuclei would have required a special classification, but no such cells were found. The remaining epithelial types listed in Table 1 were found inside, or, in the case of stratified squamous keratinizing epithelium (Fig. 6), on the surface of the fibro-muscular mass of the bladder wall. Cartilage masses within the bladders (Fig. 7) were also listed, although their relation to the original epithelium, if any, is not known. Many of the embryonic bladders recovered had their lumen filled with necrotic material and leucocytes. If they contained any epithelium, it was usually a primitive type displaying varying degrees of necrosis (Fig. 8). Such epithelia are not listed in Table 1.

In summary, transplants of bladders from 14 to 15 day old embryos, in which the epithelium survived, had embryonic transitional epithelium in 9 cases, adult transitional epithelium in 3 cases, and other epithelia or cartilage in 11 cases. In contrast, bladders from 16 or 17 day old embryos did not have any healthy

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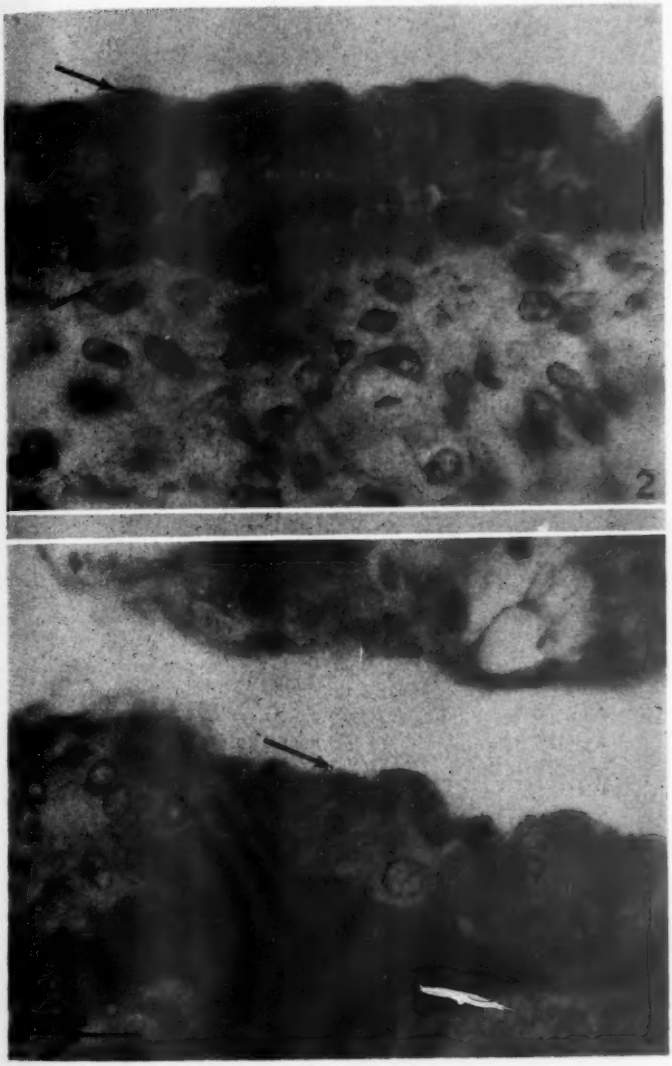
FIG. 2. Tissue adjacent to the lumen of a bladder 24 days after being transplanted from a 14 day old embryo. The extent of the epithelium is outlined by arrows. It shows no evidence of having changed its morphology since the time it was removed from the embryo. Stained with hematoxylin and eosin.

FIG. 3. Epithelium and connective tissue of bladder 17 days after being transplanted from a 15 day old embryo. The epithelium (between arrows) resembles that of a 15 day old embryo bladder except for some flattening of the surface cells and nuclei. Stained with hematoxylin and eosin.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embryonic type transitional epithelium at time of recovery, but did have adult type transitional epithelium in 11 cases. In addition, other epithelia were found in 3 of these bladder transplants (Table 1).

Discussion

The purpose of this experiment was, firstly, to see if the development of polyploidy in transitional epithelium could be prevented by transplanting embryonic bladders at a stage prior to the time at which polyploidy normally arises. Secondly, if the development of polyploidy was blocked, to see if this would affect the histogenesis of the epithelium.

Polyploidy in mouse urinary bladder epithelium normally arises in 16 day old embryos (4). When bladders from 14 and 15 day old embryos were transplanted into a subcutaneous location in adult mice, there was no sign of polyploid nuclei in most cases (Figs. 2, 3). Furthermore, when polyploid nuclei were not present, the embryonic epithelium did not develop into adult transitional epithelium. However, in three cases (Table 1) adult type transitional epithelium appeared in transplants from 14 and 15 day old embryos. These epithelia had giant nuclei in their surface cells (Fig. 4). In Feulgen stain preparations, it was evident that the increase in nuclear size was not accompanied by a proportional decrease in Feulgen positive material per unit area (Fig. 9). Since the Feulgen technique is specific for DNA, this maintenance of unit area density with increased nuclear size could only take place if the nucleus were polyploid. (Of course, this type of rough comparison does not distinguish polyploidy from high chromosome number aneuploidy. However, the chromosomal constitution of normal surface cells has been investigated by microspectrophotometric measurements on Feulgen stained nuclei and by chromosome counts, and found to be essentially

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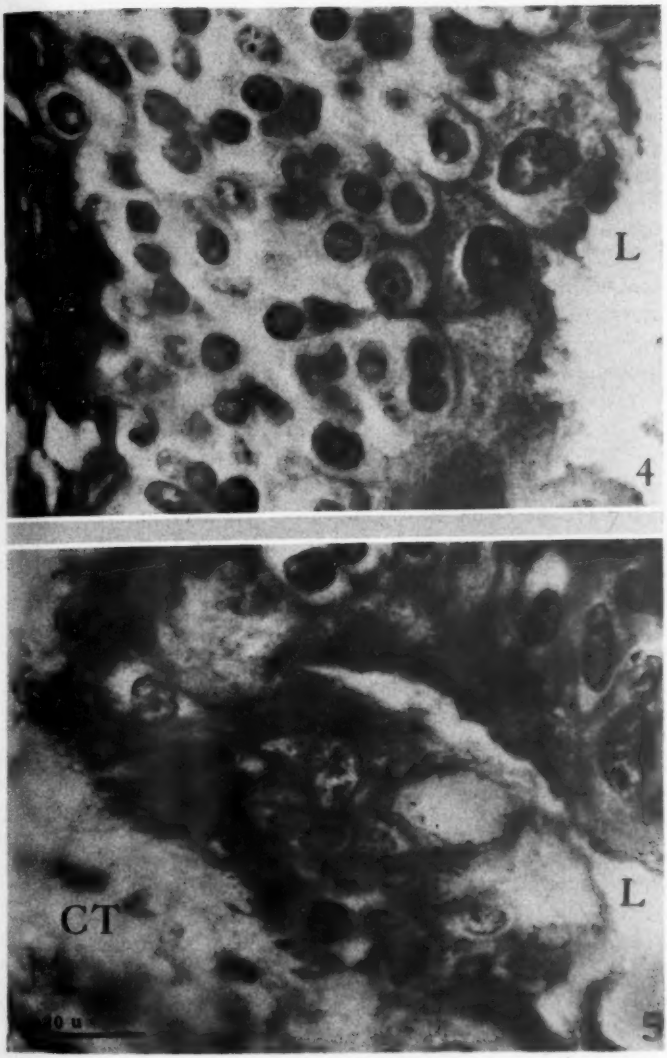
FIG. 4. Epithelium of a bladder 24 days after being transplanted from a 14 day old embryo. The surface cells, adjacent to the lumen (L), are very large with giant nuclei and periodic acid Schiff positive cytoplasm. Stained with hematoxylin and periodic acid Schiff.

FIG. 5. Epithelium and connective tissue (CT) of a bladder 18 days after being transplanted from a 17 day old embryo. The surface cells, adjacent to the lumen (L), are very large with giant nuclei and periodic acid Schiff cytoplasm. Stained with hematoxylin and periodic acid Schiff.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euploid, so the assumption of euploidy in the transplants seems warranted). Thus, bladder epithelium from 14 and 15 day old embryos gives rise to adult type transitional epithelium only when polyploid nuclei appear. Since no polyploid nuclei were present at the time of transplantation, they must have developed after transplantation. There is no theoretical reason for believing that polyploid nuclei should not arise after transplantation, and it is fortunate, for the purposes of analyzing the effects of polyploidy, that they actually did arise in a few cases.

Polyploid surface cells are normally present in the bladder epithelium of 16 and 17 day old embryos (4). When epithelia survived in transplants of these older bladders, they did not remain in an embryonic state but differentiated into adult type epithelium morphologically (Figs. 5, 10), and usually functionally also by forming cysts, as did the adult bladder transplants.

The three cases of columnar epithelium listed in Table 1 for transplants from 16 day old embryos present the same problems of interpretation as do the other epithelia and cartilage masses that were found in transplants from younger embryos. They may have arisen from the embryonic bladder epithelium or else may have grown in to replace bladder epithelium that died before the host tissue vascularized the transplant. Considering the large number of these other tissues in transplants from 14 and 15 day old embryos, it seems likely that at least some of them were derived from the primitive bladder epithelium.

In general, primitive bladder epithelium cannot produce adult type transitional epithelium after transplantation unless taken from an embryo 16 days old or older. Therefore, some change must take place in the epithelium at 16 days to give it this potentiality. Development of polyploidy is one of the events which occurs at this time. Also, epithelium from 14 day old embryos can develop into transitional epithelium if polyploidy arises in

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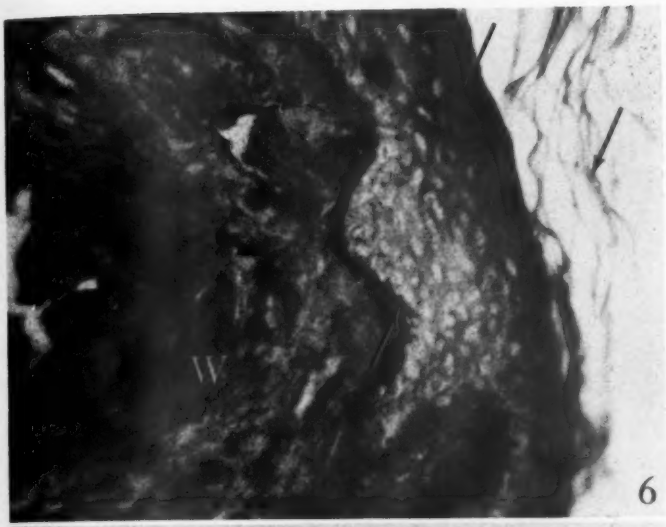
FIG. 6. Stratified squamous keratinizing epithelium (arrows) on the fibromuscular wall (W) of a bladder 19 days after being transplanted from a 15 day old embryo. When followed in serial sections, the stratified squamous epithelium was found to be continuous with a primitive type of epithelium within the bladder (to the left in photo). Stained with hematoxylin and periodic acid Schiff.

FIG. 7. Part of a cartilage mass within a bladder 28 days after being transplanted from a 15 day old embryo. Stained with hematoxylin and eosin.



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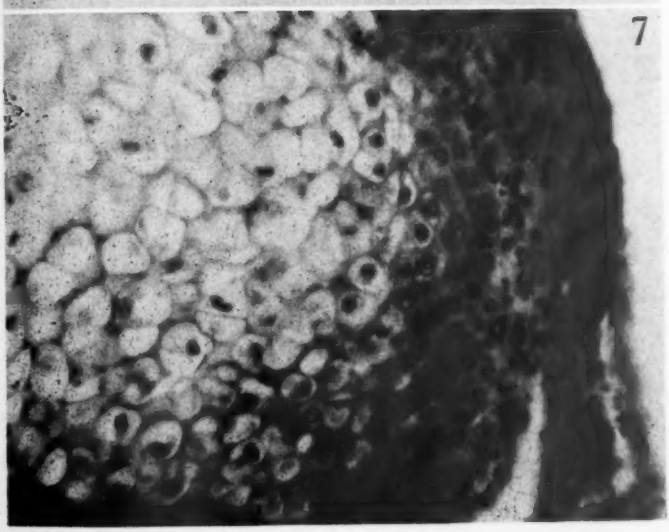

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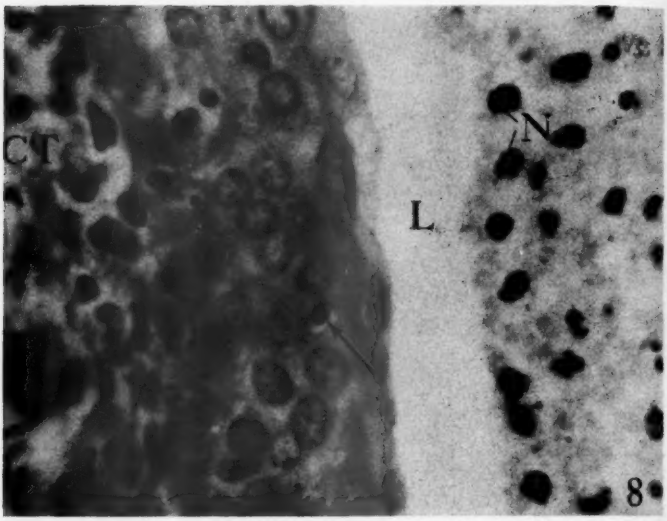

FIG. 8. Epithelium, connective tissue (CT), and lumen (L) of a bladder 13 days after being transplanted from a 16 day old embryo. The lumen (L) is filled with a mass of necrotic material and neutrophils (N). The epithelium also shows signs of necrosis (arrow) and has the primitive features typically found in embryonic bladder transplants undergoing necrosis. Stained with hematoxylin and eosin.

some of its cells after transplantation. Therefore, it is probable that the critical event in the histogenesis of transitional epithelium, which normally takes place in 16 day old embryos, is the development of polyploidy.

Summary

Embryonic urinary bladders and slices of adult bladder were transplanted subcutaneously into adult mice. The transitional

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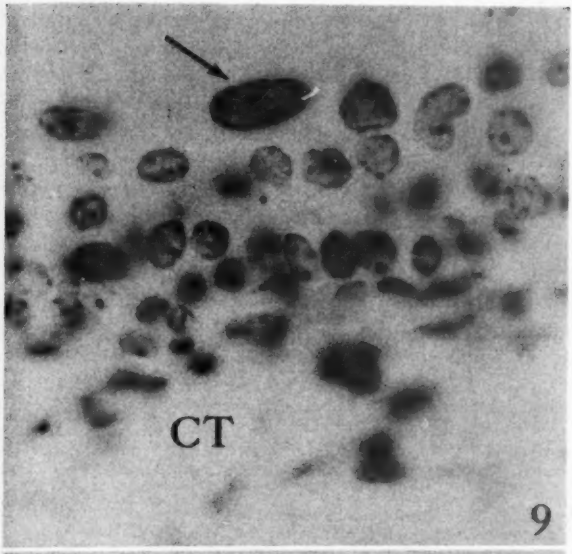
FIG. 9. Epithelium and connective tissue (CT) of a bladder 24 days after being transplanted from a 14 day old embryo. The giant nucleus (arrow) at the lumen has at least as much Feulgen positive material per unit area as do nuclei in the deeper layers of the epithelium. Stained by the Feulgen technique.

FIG. 10. Epithelium and connective tissue (CT) of a bladder 22 days after being transplanted from a 16 day old embryo. The total amount of Feulgen positive material in the surface cell nucleus (arrow) is greater than in cells of other layers in the epithelium. Stained by the Feulgen technique.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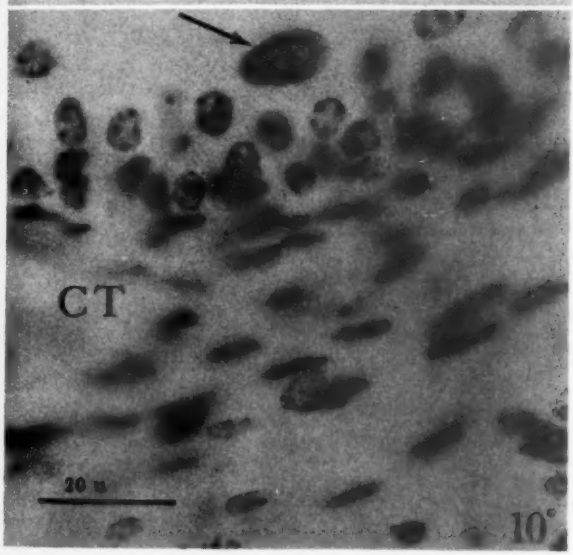
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epithelium of adult bladder slices usually formed cysts. Bladders from 16 and 17 day old embryos also tended to form cysts if the epithelium survived. This epithelium, which contained polyploid nuclei in its surface cells, developed into the adult type of transitional epithelium. Bladders from 14 and 15 day old embryos, which did not contain polyploid nuclei, did not form cysts and did not develop the adult type of transitional epithelium; except in three cases where polyploidy developed in the epithelium after transplantation. This correlation between the presence of polyploid nuclei and the ability of embryonic bladder epithelium to develop into transitional epithelium supports the hypothesis that polyploidy is essential to the differentiation of surface cells in transitional epithelium.

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FAILURE OF SODIUM SUCCINATE TO RELIEVE HYPOGLYCEMIC COMA

E. T. Richey and W. P. Wilson

The therapeutic utilization of various carbohydrate metabolites has periodically been of interest to psychiatrists. Reports of the therapeutic utilization of lactic acid for depressions[7], glutamic acid for mental deficiency[1], and succinic acid in depression[2] and confusional states[10] have appeared in the literature. Reports in the literature as to the analeptic effects of succinate in barbiturate poisoning and anesthesia[10,5,6,4] have been conflicting although the number of negative reports seem to be in the majority. Trautner, Trethwithie and Gershion[10] thoroughly investigated the effects of this substance in both animals and man. They demonstrated an analeptic effect of succinate in barbiturate coma, and a "waking up effect" in hypoglycemic coma. As well they observed a decrease in the post treatment confusion of patients who had received insulin coma or electroshock treatments. As our clinical experience in post treatment confusional states was contrary to the experience of these authors, we have attempted to repeat with modification the experiments done with patients in hypoglycemic coma. It was felt that these experiments would offer evidence as to the ability of the central nervous system to utilize succinate as a substrate in the carbohydrate cycle. We further made an effort to determine whether the "waking up" effects observed by these authors were the result of conversion of succinate to glucose by the liver.

Method

Eight physically normal patients on the psychiatric service of the University of Texas Medical Branch Hospitals, Galveston, Texas, were chosen for this study. Three of the patients were male and five were female. A total of eleven studies were done on the eight patients. Each patient was undergoing insulin coma therapy and at the time the experiments were carried out had received ten or more treatments. On the morning of the experiment, after the patient was in a stage III coma, four electroencephalographic electrodes and the electrodes for Lead II of the electrocardiogram were applied. Continuous electroencephalograms and electrocardiograms were recorded throughout the entire experiments using a Grass P-5 Polygraph. Serial venous blood

TABLE 1

Patient	Control	5–6 gm. succinate	10–12 gm. succinate	10 gm. glucose
M.J.	32.6	33.8	35.5	...
D.S.	16.3	18.9	22.2	...
T.H.	14.6	...	16.6	221
D.S.	17.1	18.4	20.8	...
M.S.	25.7	23.1	25.7	...
G.B.	14.2	12.2	...	196.1
W.H.	17.0	20.1	20.1	...
R.C.	5.7	4.1	6.2	...
M.J.	22.0	25.6	29.2	100.5
M.H.	9.8	13.3	23.5	...
M.J.	18.5	19.0	24.2	207.4
Mean	17.7	18.9	22.4	181.0

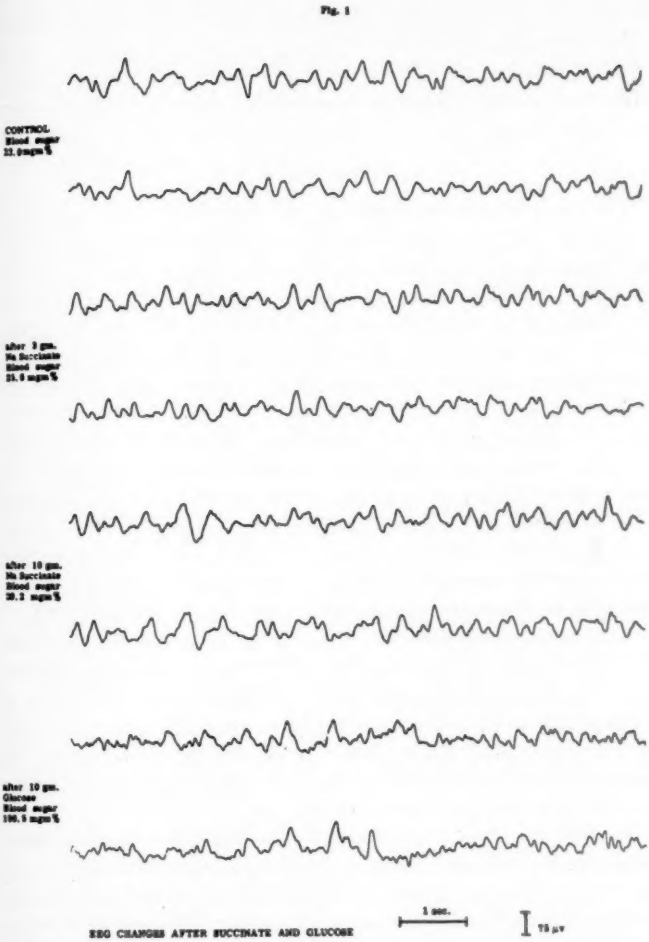
sugar levels were drawn from a convenient vein. After a control blood sugar had been drawn and a satisfactory EEG and EKG tracing obtained, 5 or 6 grams of sodium succinate in a 30% solution was administered. A rest period of five minutes was allowed to elapse and a second blood sample was obtained prior to the administration of an additional 5 or 6 grams of succinate. The patient was then observed for five to ten minutes and a third blood sample was drawn. At this point ten grams of glucose in a 50% solution was administered. A fourth blood sample was obtained in four experiments. After a five minute period of observation another 15 grams of glucose was administered and the experiment terminated.

Blood sugar levels were determined using Nelson's modification of the Somogyi method.[9].

Results

The previously described observations as to the changes in respiration, pulse, and the occurrence of flush were confirmed. Yet in not one single instance were we able to demonstrate a change in the level of consciousness or an improvement in the electroencephalogram following the injection of either 5 or 10 grams of sodium succinate. Many of the patients did become restless when the succinate injection was begun but no more so than is usual with any prolonged uncomfortable stimulus. In contrast, with an equal quantity of glucose (10 grams) all patients showed a gross increase in the level of consciousness and a reversion of the EEG to near normal.

No electrocardiographic changes were observed following the administration of succinate.



EEG CHANGES AFTER SUCCINATE AND GLUCOSE

Observations were concomitantly made on the duration of post-treatment confusion. One patient who had been subject to the prolonged period of confusion following termination of insulin coma did, at the time of the first experiment, show a decreased duration of confusion. In two subsequent repeat experiments, however, succinate did not decrease the duration of the con-

fusion. No reduction in confusion was observed in any of the other patients in this series.

Blood sugar levels did not change appreciably. A very slight statistically insignificant rise was observed for the entire group (4.7 mgm.%).

Discussion

The observations reported here do not confirm the findings of Trautner et al[10]. The utilization of doses of succinate 4 to 7 times as large as those used by the earlier investigators were calculated to produce a much more profound change in the level of consciousness of patients in hypoglycemic coma than had been the case in their series. Our decision to use the larger dosages was based on the observation of Himwich[3] that at least 8 grams of glucose is necessary to increase appreciably the level of consciousness of a patient in hypoglycemic coma. The amounts used were in excess of this quantity. Furthermore it would seem that succinate does not appreciably potentiate the effect of glucose since in none of the patients was there a gross change in the electroencephalogram before 10 grams of glucose injected over a 5 minute period were given. Unfortunately graded doses of glucose were not administered to clearly corroborate this impression.

The lack of significant increases in blood sugar levels 5 minutes after the administration of succinate would indicate that the substance is not rapidly converted by the liver. A mean increase of 4.7 mgm.% would indicate that gluconeogenesis using succinate as a substrate probably does take place. It is conceivable that determinations of arterial sugar levels would have demonstrated a greater increase in the levels of glucose.

Maddock et al[8] have administered succinate to hepatectomized dogs who had electroencephalographic changes characteristic of hpyoglycemia. No changes in the EEG were observed with succinate, in contrast to the effect to glucose which restored the tracing to normal. The findings reported here are in keeping with these observations made in animals.

Summary and Conclusions

1. Ten to twelve grams of sodium succinate were administered to 8 patients in hypoglycemic coma on 11 occasions.
2. Electroencephalographic and electrocardiographic changes

secondary to the hypoglycemia were not reversed by sodium succinate.

3. No changes in levels of consciousness were observed.

4. Sodium succinate is incapable of effectively serving as a substrate in the carbohydrate metabolic cycle of the brain of man in acute hypoglycemia.

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[b] This work was supported by a Grant from The Hogg Foundation of The University of Texas.

Immunization Information for International Travel. *Anon.* U. S. Dept. of Health, Education and Welfare, Public Health Service Publication No. 384, Revised June, 1959. Pp. 75. Supt. of Documents, U. S. Gov't Printing Office, Washington 25, D. C. $0.30.

This concise booklet covers required documents and immunizations, recommended additional immunizations, where immunizations may be obtained, detailed information on U. S. requirements, a list of fussinesses of other countries, designated yellow fever vaccination centers, restrictions on non-immunized pets, and valuable, generously conceived maps of infective areas. The information contained in this booklet, complete to the extent of serving prospective visitors to Zanzibar, Angola, the Comoro Islands, Fernando Po, Ifni, Madagascar, São Tomé and Principe, Swaziland, Togo and Alaska, is elegantly complete for both requirements and recommendations. This booklet is invaluable as a guide to friends traveling. It is worth $0.30.

G. A. Emerson

Mosquitoes of Medical Importance. Richard H. Foote and David R. Cook. Agr. Handbook No. 152, Agr. Res. Service, U. S. Dept. Agr. Pp. 158. 68 fig. Supt. of Documents, U. S. Govt. Printing Office, Washington 25, D. C. July, 1959. $2.50.

"Success in controlling arthropod-borne diseases during military operations is frequently dependent on the rapidity with which a situation can be correctly evaluated." This fact-packed quarto, subsidized by funds from the U. S. Dept. of the Army, well meets its aim in presenting ". . . the type of reference material needed by military preventive-medicine units in the field," and contributes to paramedical literature in the neglected field of geographical medicine. Its faults, if they may so be considered, are intrinsic in any such effort. "Medical importance" is considered chiefly in regard to field-authenticated human infections, and there with a high degree of critical judgment. Fowl pox, anaplasmosis, lymphocytic choriomeningitis, myxomatosis and Russian spring-summer viral infections, rickettsial infections,

tularemia, and bacterial, portozoan and fungal diseases in which mosquitoes have been implicated as possible vectors from laboratory infections, are not considered. Secondly, faults inherent in Earl B. McKinley's classic *A Geography of Disease* (George Washington Univ. Press, Washington, D. C., pp. 492, 1935) and tactfully acknowledged by him (". . we must offer apologies for certain deficiencies which could not be helped") are similarly recognized; e.g., by arbitrarily considering 35 regions ". . defined in part by the number of medically important species that could be conveniently included . ." Until greater uniformity of quality of data from different parts of the world is available, geographical medicine can deal only with practical approximations.

An overly-concise (4 p.) description of certain mosquito-transmitted diseases—malaria, yellow fever, dengue, the encephalitides, other virus diseases and filariasis—precedes the magnum opus of more than 100 pp. given over to geographical distribution, relative medical importance and 68 lucid pictorial keys ". . designed to eliminate from consideration all species that are not medically important and those that are not known to be present in a region" for the various regions, with brief discussion of physical features and distribution of diseases in those regions. The pictorial keys in this section permit rapid identification of larval and adult female forms. Following this, 37 pp. are devoted to brief discussion of bionomics, relation to disease and recorded distribution of each of some 112 species. There are 357 references to the pertinent literature.

It may well be as frustrating to the reader as it must have been to the authors, to have so many dangling loose-ends necessitated by the narrow scope of this effort. Chemical, biologic and engineering control methods are not noted; ecologic maps are absent; fundamental questions in behavior of mosquitoes (anthropophilism, endophilism, adaptability to breeding sites) are described rather than discussed; serologic surveys to delimit infective ranges are hardly noted; statements such as that malaria ". . is almost nonexistent in America north of Mexico" are not applicable to important small loci such as E. Texas; documentation of dogmatic statements is not a perfect substitute for brief presentation of supporting data, in tabular form if necessary; and the index is solely taxonomic and geographic. Provocative

statements abound without being requited, as in the differential maturation of *Wuchereria* spp. in different mosquitoes, and in the "mystery" of the relative absence of yellow fever in the Orient, ". . where dengue may in some way actually replace that disease there." By contrast, within the purely entomologic realm, a competent unraveling of the difficult *Anopheles maculipennis* complex is given.

The above criticisms may be levelled at any work which purports only to be a good descriptive working-manual in an important field; e.g., Stitt's *Manual*. The information conveyed by the present monograph is mainly available *in extenso* in William R. Horsfall's *Mosquitoes, Their Bionomics and Relation to Disease* (pp. 723, The Ronald Press Co., N.Y., 1955, $16.00), but without the primarily geographic approach, the identification keys, or any figures. For the purpose intended and the serendipitous wider interest involved, the present work is competent in execution and courageous in concept. *G. A. Emerson*

Kritchevsky, David: Cholesterol. New York, John M. Riley and Sons, Inc., 1958, 291 pp. Appendex. Index.

This book by an Associate Member of the Wistar Institute and Assistant Professor of Biochemistry in Medicine at the University of Pennsylvania is a summary of the work of his group as well as a rather complete compilation of data in reference to this enormously pursued subject. Synthesis and derivatives, absorption and transfer, the metabolic pool and its side- and end- products, cholesterol in disease, the blood cholesterol, and analytic methods, are well covered in successive chapters.

The appendix contains physical constants, cholesterol content of foods and tissues and serum levels in various animals. As minor criticism, the references of the blood cholesterol chapter number 546, are somewhat limited to West European and American literature. The list of hypo-cholesterolemic measures is perhaps inevitably inadequate because of rapid advances in this field and the purely biochemical, and not clinical, bent of the author.

The book satisfies the need of summarizing the present knowledge of the subject of cholesterol and its metabolism and should be of great interest to research and clinical workers in the fields of fat nutrition and metabolism, atherosclerosis and allied states, comparative biochemistry and geographical medicine among others. *Arthur Ruskin*

CALLING ATTENTION TO

New Year 1960

Preclinical: E Adams offers hypotheses on mechanisms of toxic action (*Sci Amer* 201/5/76/Nov/59). J Axelrod surveys metabolism of sympathomimetic amines (*Physiol Rev* 39/751/59). A Bouhnuys & G Lundin describes distribution of inspired gas in lungs (*ibid* p 731). CJ Carr reviews pharmacology of Psychomimetic drugs (*Internat Rec Med* 172/702/59). R Charlier's Belgian Cardiology Soc Prize Report describes ethyl-diiodo-hydroxy-coumarone ("Amphivix") as intense long acting safe coronarodilator (*Acta Cardiol* Suppl VII, 1959, 60 pp). WE Culver makes detailed annotated bibliography of effects of cold on humans (*Physiol Rev*, Suppl 3, October 1959, 524 pp). WH Daughaday describes steroid-protein interactions (*Physiol Rev* 39/885/59). RM Featherstone & A Simon edit *A Pharmacologic Approach to the Study of the Mind*, a symposium at the Univ of California Medical Center, San Francisco (CC Thomas, Springfield, Ill, 1959, 399 pp, $10). HF Harlow describes development of learning in rhesus monkeys (*Amer Sci* 47/459/59). B Holmstedt discusses organophosphorus cholinesterase inhibitors as insecticides & nerve gases (*Pharmacol Rev* 11/567/59). AG Karczman & JH Howard find anorexigenic action in methylphenidade & Pipradrol similar to the amphetamines (*Proc Soc Exp Biol Med* 102/163/59). Y Kase & Co report potent nonnarcotic safe antitussive, methyl-dithienyl-methylene piperidine (*Chem Pharmaceut Bull* 7/372/59). PI Korner notes circulatory adaptations to hypoxia (*Physiol Rev* 39/687/59). JC Krantz & Co find trichloroethane a volatile liquid inhalation anesthetic like chloroform (*Anesth* 20/535/59). HS Lawrence discusses homograft sensitivity (*Physiol Rev* 39/811/59). WT Lippincott opens symposium on biochemistry in relation to general chemistry (*J Chem Educ* 36/534–554/59). DM Little reviews hypothermia with 428 references (*Anesth* 20/842/59). S Loewe develops isobolographs to show effects of drug combinations (*Arzneimittel-Forsch* 9/449/59). W Modell discusses drug evaluation in human subjects (*J Pharm Pharmacol* 11/577/59). M Sidman opens new journal with discussion of behavorial pharmacology (*Psychopharmacol* 1/1/59). SE Wright analyses *The Metabolism of Cardiac Glycosides* (CC Thomas, Springfield, Ill, 1959, 144 pp, $5).

Clinical: G Baehr & P Klemperer edit neat survey of *Systematic Lupus Erythematosus* (Grune & Stratton, New York 16, 1959, 90 pp, $4). DY Hsia describes *Inborn Errors of Metabolism* (Yearbook Publ, Chicago 11, Ill, 1959, 358 pp, $9.5). V Herbert describes *The Megaloblastic Anemias* (Grune & Stratton, New York 16, 1959, 168 pp, $6). HG Mandel describes fate of anticancer compounds (*Pharmacol Rev* 11/743/59). JH Menkes, who first noted "Maple-Syrup Disease" (*Pediatr* 14/462/54), now describes its characteristic early cerebral degeneration with accumulation of keto-acids inhibiting synthesis of cerebronic acids (*Neurol* 8/826/59). JP Merrill surveys transplantation of normal tissues (*Physiol Rev* 39/860/59). CC Seltzer correlates nonsmoking with masculinity (*Science* 130/1706/Dec 19/59). JH Shaw surveys agents inhibiting caries (*Pharmacol Rev* 11/705/59). MW Williams finds aspirin best for ischemic pain (*Toxicol Appl Pharmacol* 1/590/59). S Wolf

well analyses the pharmacology of placebos (*Pharmacol Rev* 11/689/59).

General: M Clagett edits *Critical Problems in the History of Science* (Univ Wisconsin Madison 6, 1959, 322 pp, $5). PF Clark gives fine account of microbiologist Theobald Smith (1859–1934) (*J Hist Med* 14/490/59). Sir Weldon Dalrymple-Champneys considers wives of famed English physicians (*Proc Roy Soc Med* 52/937/59). JM Dorsey & WH Seegers recommend *Living Consciously: The Science of Self* (Wayne State Univ Press, Detroit 2, Michigan, 1959, 184 pp, $5). LS Kubie asks whether preventive psychiatry is possible (*Daedalus* 88/646/59). HJ Muller notes prospects of genetic change (*Amer Sci* 47/551/59). B Russell intrigues as usual in *My Philosophical Development* (Simon & Schuster, NY, 1959, 189 pp, $4). Disgusting details about Hollywood's worst are given by AR St John in *First Step Up Toward Heaven*, a nauseous account of Forest Lawn Memorial Park (Prentice-Hall, New York, 1959, 293 pp, $5). Informative is Homer Smith's historical account of renal physiology (*Bull Georgetown Univ Med Center*, 13/4/59). RF Sognnaes reflects on development of preventive concept in century of Amer Dental Asso (*Science* 130/1681/Dec 18/59). SS Stevens proposes quantifying sensations by power principle, a sort of mathematical necessity (*Daedalus* 88/606/59). EJ Van Liere pleasantly recounts how he, *A Doctor Enjoys Sherlock Holmes* (Vantage Press, New York, 1959, 141pp, $3). JT Wilson (Pres Internat Union Geodesy & Geophysics) tells about Red China in *One Chinese Moon* (Hill & Wang, New York 11, 1959, 267pp, $5).

Science Study Series: Doubleday & Co., Garden City, NY, with Wesleyan University Press, Columbus 16, Ohio, are publishing new books in paper-back editions covering current science: good reading for staff, students & intelligent public; IB Cohen, *The Birth of a New Physics: From Copernicus to Newton*, 1960, 200pp, 95c; DG Fink & DM Lutyets, *The Physics of Television*, 1960, 160pp, 95c; A Holden & P Singer, *Crystals & Crystal Growing: Order in Nature*, 1960, 320pp, $1.45: WA Van Bergeijk & JR Pierce, *Waves and the Ear: What We Hear and How*, 1960, 235pp, 95c. Bargains!

Preclinical: J Bjerksten & Co show immobilization of proteins in whole body irradiation, (*J Amer Geriat Soc* 8/37/60). JHV Brown & Co attempt intracellular localization of anterior pituitary hormones (*Endocrinol* 66/1/60). RH Buller & CJ Carr study organic nitrate blood pressor depressor (*Arch Internat Pharmaco* 123/452/60). C Coers & Al Woolf cover *The Innervation of Muscle* (CC Thomas, Springfield, Ill, 1960, 149pp, $9). W Goody discusses time & the nervous system, the brain as a clock (*Lancet* 2/1155/Dec 26/59). B Minz well surveys role of adrenergic factors in the physiology & pathology of the central nervous system (*Biol Med* 48/577–645/59, with 160 ref). CM Pomerat notes rhythmic contractions of Schwann cells (*Science* 130/1759/Dec 25/59. Our E Retzlaff & J Fontaine show chemical correlation in central nervous system to reciprocal inhibition (*ibid* 131/104/Jan 8/60). MJH Smith reveals uncoupling of oxidative phosphorylation reactions by salicylates (*J Pharm Pharmacol* 11/705/59). Al Shlyakhman & Co study distribution & metabolism of C^{14} procaine (Probl Med Chem USSR 5/145/59).

Centennial of Alexander von Humboldt's Death: 1859, centennial of *Origin of Species by Means of Natural Selection* by Charles Darwin (1809–1882) ushered in new era of evolutionary concepts, and closed era of great naturalist

explorers in death of von Humboldt (1769–1859). KE Rothschuh introduces symposium evaluating Humboldt's work (*Sudhoffs Arch Gesch Med Naturwiss* 43/97–190/59), with discussion by H Schipperges on Humboldt's medical outlook. EF Dolan looks into *Green Universe: The Story of Alexander von Humboldt* (Dodd Mead, New York, 1959, 244pp, $3.5).

Clinical: EW Baeder describes self-poisoning with lead (*Indust Med Surg* 29/1/60). DB Dill & KH Jacobson describe toxicity of propellant fuels & oxidizers (*Armed Forces Med J* 11/125/60). AP Freeman & HH Merritt edit worthy volume on *Headache: Diagnosis & Treatment* (FA Davis, Philadelphia, 1959, 401pp, $8). LP Garrod introduces one of those fine English symposia, *Antibiotics in Medicine* (*Brit Med Bull* 16/1–101/Jan 1960). E Koenig compiles bibliography on *Maternal Disorders Related to Fetal Stress, Perinatal Death & Congenital Defects* (Public Health Service Publ 669, Publ Health Bibl Series 25, US Govt Printing Office, Washington DC, 1959, 33pp, 15¢). GW Larimore reports on British narcotic system which may not be workable in USA (*NY State J Med* 60/107/60). PAG Monro reports well on *Sympathectomy: An Anatomical & Physiological Study with Clinical Applications* (Oxford Univ Press, London, 1959, 290pp, $17.5). WE Nance gives introduction to gemellology (twins) (*Med* 38/403/59). H Narabayashi & Co find procaine-oil-wax pallidomy helpful in infantile cerebral palsy (*Neurol* 10/61/60). H Popper & F Schaffner describe drug-induced hepatic injury (*Ann Inter Med* 51/1230/59). Note special Symposium on the thyroid (*J Clin Endocrinol & Metab* 20/1–135/60).

General & Historical: A Beck discusses English anti-pollution legislation (*J Hist Med* 14/475/59). AH Dupress well biographizes *ASA Gray* (1810–1888), Darwin's influential American botanist friend (Belknap Press, NY, 1959, 505pp, $7.5). HJ John well describes Jan Evangelista Purkinje (1787–1869), great Czech physiologist, a scientist & patriot (*Mem Amer Philosoph Soc*, 49/94/1959). HC Lehman studies age in relation to achievement in chemistry (*Geristrics* 15/19/60). D Lerner edits colloquium on quantity & quality (*Daedalus* 88/575–645/59). KE Rothschuh offers *Theorie dee Organismus: Bios, Psyche, Pathos* (Urban &Scharzenberg, W Berlin, 1959, 345pp, DM 48). JR Royce goes on search for meaning (*Amer Sci* 47/515/59). AC Sherwin relates musical creativeness and emotional disturbance (*Bull NY Acad Med* 36/55/60). V Starrett tells about *The Private Life of Sherlock Holmes*, a reprint for Baker St. Irregulars (Univ Chicago Press, Chicago 37, 1960, 176pp, $5). AC Vaughan recreates ancient Knossos in *The House of the Double Axe* (Doubleday, Garden City, NY, 1959, 310pp $6). Greer Williams tells about *Virus Hunters* (AA Knopf, NY, 1959, 522pp, $6). E Wilson revives *Memiors of Hecate County* (LC Page, NY, 1960, 447pp, $6).

Clinical: DK Adler edits *Journal of New Drugs*, a bimonthly with no ads, 660 Madison Ave., New York 21, $10 a year. HH Anderson surveys new drugs in amebiasis as glycobiarsol, some acetamides, phenanthrolines & diamines (*Clin Pharmacol Therap* 1/78/60 RS Atkinson reviews trichlorethylene anesthesia (*Anesth* 21/67/60). AE Bennett & Co note brain damage from chronic alcoholism (*Amer J Psychiat* 116/705/60). HW Brown reviews actions & uses of anthelminitcs (*Clin Pharmacol Therap* 1/87/60). ED Fries analyses hemodynamics of hypertension (*Physiol Rev* 40/27/60). RP Geyer

surveys value of parenteral nutrition (*nbid* p150). P Gyorgy opens symposium on protein requirements in humans (*Fred Proc* 18/1025/59). ER Harrell & FC Bocabo review treatment of systemic fungus diseases (*Clin Pharmacol Therap* 1/104/60). BL Vallee & Co describe magnesium deficiency syndrome (*New Eng J Med* 262/155/Jan 28/60).

Atherosclerosis: NP Larsen & W Bortz show coronary atherosclerosis at post-mortem may be correlated with diet, low in Japanese & Chinese & high in Hawaiian & Caucasian (*Haw Med J* 19/159/59). GP Wright chairs broad symposium on atherosclerosis (*Proc Roy Soc Med* 53/9–40/60).

General: HT Brown considers patents in pharmacy & medicine (*J Pharm Pharmacol* Suppl Vol 11, Dec, 59). AG Foraker gives biting parable of 3 acolytes in medical research (*New Eng J Med* 262/81/Jan 14/60). R Forreger comments on John Snw (1813–1858) & his studies on toxicity of CO_2 (*Anesth* 21/20/60). WH Kessenich states Food & Drug Administration policies in regulating new drugs (*Clin Pharmacol Therap* 1/53/60). B Roueche does neat job of socio-science writing in series on alcohol (*The New Yorker* Jan 9, 16, 23/60). R White tells story of determined skill in *Yankee from Sweden:* The Dream & the Reality in the Days of John Ericsson (Holt, NY, 60, 299pp, $4.5).

PreClinical: A Bishop considers drug resistance in protozoa (*Biol Rev Cambridge Philos Soc* 34/445/59). J Brozek & W Kinzey analyse age changes in skinfold compressibility (*J Gerontol* 15/45/60). J Crossland neatly reviews chemical transmission in the central nervous system (*J Pharm Pharmacol* 12/1/60). Sir Charles Darwin doubts that humans can control their numbers: watch out! (*Perspect Biol Med* 3/252/60).

SF Epstein & Co report dynamic states of adenosinetriphosphate (ATP) in muscle (*Ukran Biochem J* 31/815,826/60). EA Evans Jr considers viruses in relation to evolution (*Perspect Biol Med* 3/213/60). WO Fenn introduces discussion on life in space (*Fed Proc* 18/1241/59). H Gaffron pleasantly discusses the origin of life (*Perspect Biol Med* 3/163/60). I Gersh & HR Catchpole discuss nature of ground substance in connective tissue (*Perspect Biol Med* 3/282/60). NI Grashchenkov indicates excess potassium & acetylcholine in myotonia, recommending treatment by reducing potassium intake & giving ACTH (*Neurol* 10/95/60). D Harman suggests free radical theory of aging in noting age effect on serum mercaptan (*J Gerontol* 15/38/60). FB Hershey & Co report quantitative histochemistry of human skin (*J Histochem & Cytochem* 8/41/60). H Hoagland arranges important symposium on fertility controlling steroids (*Fed Proc* 19/1039–1074/59). HC Hodge introduces good session on technical toxicology covering carbon tetrachloride, organic phosphates & heavy metals (*Ibid* 1013–1038). G Kahlson finds a place for histamine in normal physiology (*Lancet* 1/67/Jan 9/60). T Koppanyi suggests principles of comparative pharmacology (*Clin Pharmacol Therap* 1/8/60). IM London reviews netabolism of mammalian erythrocyte (*Bull NY Acad Med* 36/79/60). RE Caman & E Robbins report quantitative biochemical findings in Wallerian nerve degeneration (*J Neurochem* 5/18,32/60. O Mickelsen introduces colloquim on effects of high calcium intake (*Fed Proc* 18/1075–1124/59). WTJ Morgan gives Croonian lecture on chemical basis of blood group specificity (*Proc Roy Soc B* 151/308/60). JW Newberne & VB

Robinson comment on low incidence of neoplasms in subhuman primates (*Amer J Vet Res* 21/150/60). DW Richards is solidly realistic about dislocation & perturbations of homeostasis (*Perspect Biol Med* 3/238/60). JF Riley gives neat history of knowledge of mast cells (*Endeavor* 19/4/60). JR Robinson surveys metabolism of intracellular water (*Physiol Rev* 40/112/60). BC Saunders reviews toxic phosphorus & fluorine compounds influencing phosphorilation & transiodination (*Endeavor* 19/36/60). FB Seibert develops a theory of immunity in Tb (*Perspect Biol Med* 3/264/60). M Stacey tells exciting story of nonulosaminic acids, aminosugars involved as cell sites for virus action (*Endeavor* 19/43/60). RC Wong & IE Liener find acetylation changes trypsin action (*Biochim B Acta* 38/80/60).

CHAUNCEY D. LEAKE, *The Ohio State University*

NEWS NOTES FROM THE MEDICAL BRANCH UNIVERSITY OF TEXAS, GALVESTON

ACADEMIC TRAINING PROGRAMS FOR MEDICAL SCHOOL TEACHERS

Training in cardio-vascular research. This program under a Grant from the National Institutes of Health, is open to undergraduate as well as postgraduate students. It is an extension of a joint plan under the departments of Internal Medicine, Radiology and Pediatrics. It enters all areas of cardiovascular research, including diagnosis, care and handling of pediatric cardiovascular patients.

Training in hematologic research. The program operates under a grant from the National Institutes of Health in the amount of $26,429.00 beginning July, 1960. Participants must have completed programs for the M.D. or PhD degrees in one of the biological sciences. This is a joint venture under the departments of Internal Medicine (Hermatology and Physiology).

Training program in Anatomy. Pre- and Postdoctoral training programs in Anatomy are available to qualified students. Stipends range from $1800 to $4200 for undergraduates, starting at the $6000 level for postdoctoral fellows. Tuition and a modest travel allowance are provided for. The department has ten full time faculty members, all with active research programs. Training may include electron microscopy, tissue culture methods, phase cinematography, autoradiography, histochemistry, medical genetics, experimental endocrinology and modern techniques for investigating the nervous system. Training in other divisions of the University are provided in accord with desires and best interests of students For further information write the chairman of the department.

SPECIAL LECTURES

Jan. 16. Theodore P. Eberhard, Ann Arbor, Mich. Problems in the management of Cancer of the breast.

Jan. 26. Bruno Balke, Space Medicine Dept. USAF, Brookes Field. Topics in performance physiology.

Jan. 27. Harold Rosen. Use of hypnosis to control pain and "induce" anesthesia. Principles of trance induction, The role of hypnosis in psychotherapy.

Feb. 2. Andre Couran, Bellevue Hospital. Recent developments in physiopathology of pulmonary emphysema.

Feb. 8. Hans Stich. Saskatoon, Saskatchewan Nuclear-cytoplasmic relationships.

Feb. 8. Pieter Guillard, Leiden, Netherlands. Sigma Xi lecture. Parathyroid and bone in vitro.

Feb. 9. Julius H. Comroe. Am. Physiol. Soc. Advances in cardiopulmonary physiology.

Feb. 15. John Biesele, Austin. Chromosomes and cancer.

Feb. 23. John A. Bjöorksten. Madison Wis. A theory concerning the molecular basis of aging.

Feb. 24. Hubert Smith, Austin. Foundation principles of legal responsibility for medical malpractice.

March 11. Eugene Stead, Duke University. Meyer Bodansky lecture. Education of the doctor.

March 8. Robert Pudenz, Pasadena, Calif. The Greenwood lecture. The dynamic aspects of head injuries.

March 29. Leon Sokoloff. Nat. Inst. Health. Sigma Xi lecture. The biology and comparative pathology of joint diseases.

May 12. David A. Rogers, Vanderbilt University. McLaughlin Lecture Pulmonary complications of Asian influenza.